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THE NEW ZEALAND

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Editor: D. Cairns, M.Sc. (Assistant Editor: M. O'Connor, M.Sc.) Department of Scientific and Industrial Research, Wellington

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APPLE-MOSAIC IN NEW ZEALAND

By J. D. ATKINSON and E. E. CHAMBERLAIN, Plant Diseases Division, Department of Scientific and Industrial Research

(Received for publication, 23rd January, 1948)

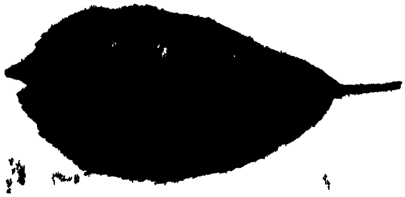
Summary

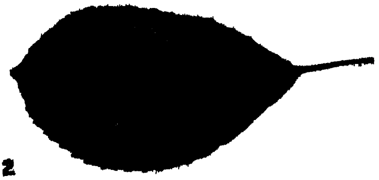
A cream and yellow mottling of apple foliage has been present in New Zealand for many years. This condition appears to be the same as that known in other countries as apple-mosaic, the causal virus of which has been listed by Smith (1937) as *Pyrus* virus 2. Infected trees have been observed in all main apple growing areas, and in several outlying orchards. The disease has been seen in 39 varieties and 3 stocks. Mosaic symptoms developed on 26 of 36 healthy apple seedlings budded with infected wood.

APPLE growers in New Zealand have known a form of yellow mottling in Jonathan foliage for many years. As this appeared to cause no injury to trees little attention was paid to it, and some growers came to regard it as a varietal characteristic. Experiments carried out over the past two years have shown that this mottling is of virus origin and is apparently identical with apple-mosaic.

SYMPTOMS

Infected apple leaves show many forms of mottling, the most common being a number of small irregular creamy or yellow spots that stand out conspicuously against the dark green of normal leaf tissue. These spots do not occur in any regular pattern, and any number from one to several hundreds may be present on a single leaf (Figs. 1 and 2). Sometimes spots are so numerous that parts of the leaf appear completely chlorotic. Where chlorosis involves a considerable area of the leaf it is common to find necrosis taking place as the season advances. More rarely bands of chlorotic tissue develop along some of the larger veins (Fig. 3). In other cases mottling may take the form of a light and dark green mosaic (Fig. 4), or of large vaguely defined patches of yellowish white tissue (Fig. 5). One or all of these symptoms may be present on a single tree, or even on a single branch. All leaves on a shoot may show symptoms, though it is more usual to find a few mottled leaves among apparently normal ones.





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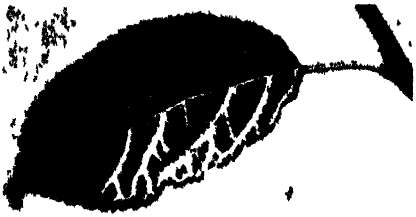

FIG. 1
Sparse mottling on Jonathan leaf infected with apple-mosaic
(× ½) Photo L. H. Wright

FIG. 2
A severe case of the spotted form of apple-mosaic on a Jonathan leaf
(× ½) Photo R. I. Hughes

FIG. 3
Bands of chlorotic tissue along veins accompanied by irregular spotting on a leaf of Golden Delicious
(× ½) Photo L. H. Wright

FIG. 4
Mosaic pattern produced by apple mosaic on Golden Delicious
(× ½) Photo L. H. Wright

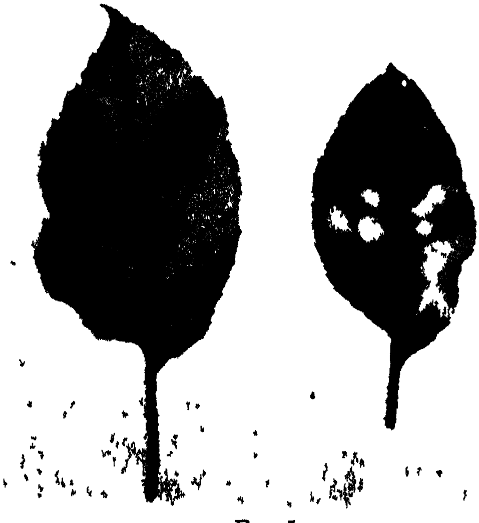

FIG. 5
Extensive chlorosis produced by apple-mosaic on artificially inoculated Jonathan seedlings
(Natural Size) Photo L. H. Wright

In commercial orchards, symptoms are often pronounced on Golden Delicious and Jonathan, it being common to find over half the leaves on infected trees of these varieties showing mottling On other varieties, symptoms may be scattered over the tree, but it is more usual to find only a few spurs showing mottled leaves. Field observations suggest that trees showing severe symptoms are less vigorous than healthy trees. No fruit symptoms have been observed. Infection has been seen on the following apple varieties:—Apple of Commerce, Afriston, Ballarat, Beauty of Bath, Charles Ross, Cox's Orange Pippin, Delicious, Dougherty, Dunn's Favourite, Elsie Grant, Giant Jeniton, Glengyle Red, Golden Delicious, Granny Smith, Gravenstein, Jonathan, Laxton's Pearmain, Laxton's Pioneer, Laxton's Royalty, Lord Derby, Lord Lambourne, Lord Wolsely, Newton Wonder, Oratia Beauty, Parlain's Beauty, Rival, Rokewood, Russet (type unknown), Scarlet Nonpareil, Scarlet Pearmain, Scarlet Queen, Shorland Queen, St. Everard, Statesman, Sturmer Pippin, Symonds Winter, Willie Sharp, Worcester Pearmain, Yates, and the Malling Stocks Nos. IX, XII and XVI.

INCIDENCE

Apple-mosaic has been found in the five main apple growing areas, namely Auckland, Hawkes Bay, Nelson, Canterbury and Central Otago. In addition it has been found in Auckland Province at Te Hana, Te Puke, Tinopai, Tuakau and Warkworth, and in Wellington Province at Levin, and Masterton. A detailed survey was made in one Nelson orchard where every tree of 80 mature Jonathans was found to show some degree of infection. In three blocks of Dougherty and six of Granny Smith examined near Auckland, between 60 per cent. and 80 per cent. of the trees were found to be infected, but very few leaves per tree showed mottling. In three blocks of Golden Delicious all trees examined showed mosaic, the percentage of leaves affected being high. No detailed examinations have been made in other districts. In two small blocks of Jonathan each of which was raised from clonal buds, every tree showed infection; but the number of leaves showing mottling, and the severity of symptoms varied widely between trees.

TABLE I. TRANSMISSION OF APPLE-MOSAIC FROM APPLE TO APPLE BY BUDDING
Budding was carried out on 15/3/46. Records taken on 25/11/46.

Budwood Source	Type of Seedling Inoculated.	No. of Seedlings Budded.	No. of Seedlings Infected.
Granny Smith	Granny Smith	4	2
	Jonathan	4	2
Jonathan	Granny Smith	8	5
	Jonathan	8	7
Statesman	Jonathan	1	1
	Sturmer Pippin	1	
	Granny Smith	1	1
Golden Delicious	Jonathan	1	1
	Sturmer Pippin	1	1
	Granny Smith	1	-
Ballarat	Jonathan	1	1
	Sturmer Pippin	1	1
	Granny Smith	1	1
Elsie Grant	Jonathan	1	1
	Sturmer Pippin	1	1
	Granny Smith	1	1

38 untreated check seedlings all remained healthy.

TRANSMISSION

Seeds collected from Jonathan, Sturmer, Pippin and Granny Smith apples were grown in pots under glass during the 1945-46 season. Seedlings grew well and remained free from virus. Budwood was collected from infected trees in the field, only those shoots showing pronounced symptoms being selected. Buds from these were inserted into healthy seedlings in March, 1946, and pots then moved outside. Early in the following spring seedlings were set out in nursery rows, but stocks were not cut back. Symptoms became visible on both scions and seedling stocks within six weeks from the commencement of spring growth. Results are set out in Table I.

At budding some seedlings were smaller in diameter than the budwood. As a result only 19 of the 36 buds grew, but despite this 26 of the seedlings developed mosaic symptoms. Thirty-eight check seedlings in an adjacent row remained healthy. Symptoms which developed on seedling stocks were variable, but it was not possible to correlate type of symptom with source of inoculum or with seedling employed. In many cases several types of symptom appeared on the one seedling. Results suggest that leaf mottling in the five scion varieties was caused by the same virus.

DISCUSSION AND CONCLUSION

Vibert working in France in 1863 is reported to have observed perpetuation of variegation on apple foliage by budding, and to have noticed its spread to leaves of the stock (Bradford and Joley, 1933). At that date, infectious viruses were not recognized as such, and the earliest record of transmission of apple-mosaic as a specific disease appears to be that of Orton and Wood (1924). Since then the transmission of apple-mosaic by budding or grafting has been reported from Michigan (Bradford and Joley, 1933), California (Thomas, 1937), Bulgaria (Christoff, 1934), Nova Scotia (Hockey, 1943), South Africa (Louw, 1944), and England (Wallace *et al*, 1944). From descriptions and figures published it is probable that all were dealing with the same virus. Christoff (1934) claimed that apple-mosaic was transmissible from apples to pears and rose haws. Thomas (1937) by budding, grafting or inarching obtained transmission from apples to *Cotoneaster harroviana*, loquat (*Eriobotrya japonica*), *Photinia arbutifolia*, and *Sorbus pallescens*. The authors cited have between them recorded mosaic in 17 named apple varieties. Smith (1937) classified apple-mosaic as *Pyrus* virus 2.

Both in symptoms and ease of transmission by budding the New Zealand virus agrees closely with descriptions of apple-mosaic (*Pyrus* virus 2).

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TOMATO-SPECK OF TOMATO

By W. D. REID, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 31st March, 1948*)

Summary

Tomato-speck, a bacterial disease of tomato, is recorded in New Zealand. The disease on fruits is characterised by small (1 mm.) dark brown pustules surrounded by a narrow circular light halo. On leaves, lesions are usually numerous and are small (1 to 2 mm.) dark brown to black dry spots surrounded by a yellow zone. Infected plants are light in colour, stunted and unhealthy in appearance.

The morphology and the cultural characters of the causal organism are described. They agree with those given for *Pseudomonas punctulans* (Bryan) Dowson.

TOMATO-SPECK is a bacterial disease of tomato caused by *Pseudomonas punctulans* (Bryan) Dowson (Dowson, 1943). The disease was first found in New Zealand in 1944 at Hastings, where it was causing serious stunting of Tatura dwarf variety in a field crop. The crop was raised from imported seed and young plants in seedling boxes showed extensive leaf infection. The disease has not become widespread in this country but is of economic importance in the United States of America and in Canada (Conners, 1942). It has also been reported from Formosa (Okabe, 1933) and Australia (Fish, 1939).

SYMPTOMS

The name tomato-speck (Bryan, 1933) was derived from the symptoms on fruits. Lesions are very small, dark, round pustules, rarely more than 1 mm. in diameter and appear on the fruit surface as specks surrounded by a white halo (Figs. 1 and 2). They do not penetrate deeply into the flesh.

On leaves lesions are at first dark water-soaked spots. Later they become dark brown, almost black, and irregular in outline but remain small, not more than 3 mm. in diameter (Fig. 3). They are dry and rough in appearance and are surrounded by a narrow yellow halo. The spots are often crowded on the leaf surfaces giving a yellow appearance to affected foliage. Where lesions occur on leaf veins infection progresses along the veins forming narrow lesions. Natural stem lesions as described by Bryan (1933) have not been observed in New Zealand.

Infected plants in the field are often markedly stunted being less than half the height of normal plants. They are unthrifty in appearance largely because infected leaves are yellow to brown in colour and small. On fruits the many lesions are unsightly and render fruits unsuitable for marketing.

CAUSE OF DISEASE

Microscopic examination of typical lesions showed the presence of abundant bacteria and isolation on beef-peptone-agar regularly gave pure cultures of the causal organism. Numerous glasshouse tests in 1945 and 1946 involving over 50 plants showed that dwarf (Tatura and Australian Dwarf) and staked (Vetomold, Potentate and Kondine) varieties of tomatoes are susceptible to infection.

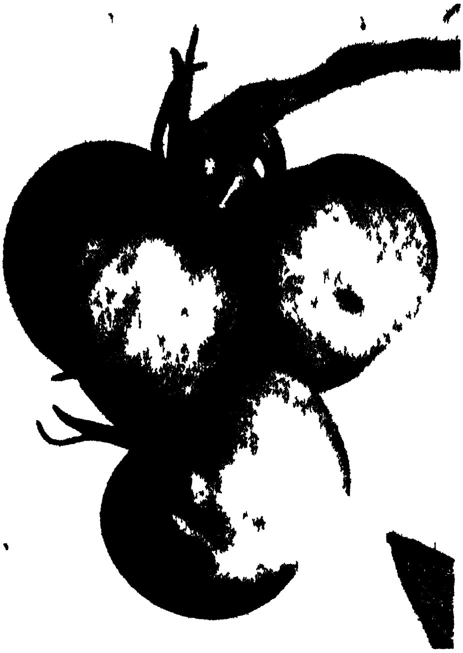

FIG. 1

Lesions on green fruit following hail injury and spray inoculation

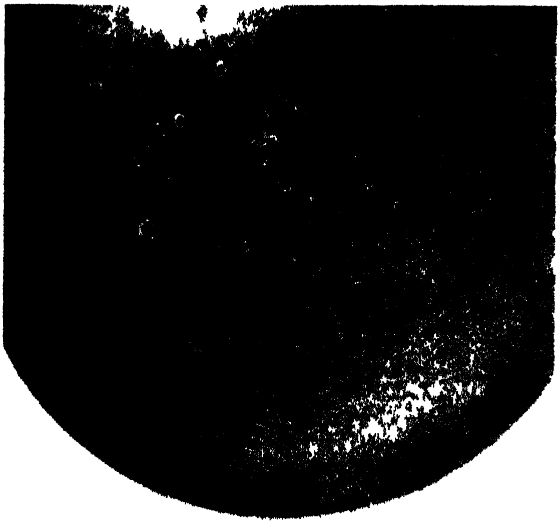

FIG. 2

Lesions on ripe fruit

Leaves, stems and fruits of plants were lightly injured by gently rubbing the surfaces with a cotton pad or flicking with a sheet of paper. The plants were then sprayed through an atomiser with a water suspension of organisms from beef-peptone-agar slopes and placed in a saturated atmosphere for 24 hours. Infection of leaves and fruits was first observed after five days as minute, black dots. These slowly increased in size to form typical lesions of the disease. Lesions were not formed on stems unless they were more severely injured by pricking or scratching. Spraying with inoculum without previous injury produced a few lesions on leaves but not on fruits or stems. Check plants injured and sprayed with water only, failed to produce obvious lesions.

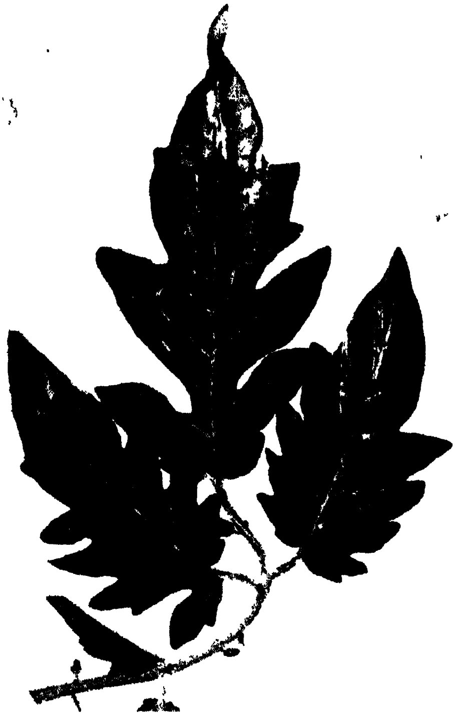

FIG. 3

Leaf Lesions following brushing surface and spray inoculation

Isolation of the causal organism from lesions induced by inoculation was readily and repeatedly obtained on beef-peptone-agar. Reinoculation of tomato plants with cultures so obtained gave typical lesion formation.

The symptoms from field specimens and from glasshouse inoculations agree with those described by Bryan (1933) for bacterial-speck of tomato.

MORPHOLOGY OF CAUSAL ORGANISM

Vegetative cells after two days growth on beef-peptone-agar exist as short rods with rounded ends, singly, in pairs and as short chains. They vary in size from 0.5 × 0.6 μ to 0.6 × 4.8 μ for single rods and to 0.6 × 16 μ for chains. Single rods average 0.5 × 1.6 μ. The organism is gram negative (Hucker stain) and is motile by one to three polar flagella (Leifson, Gray, Bailey and Morton stains), some rods show flagella with definite side origin.

CULTURAL CHARACTERISTICS*

Beef-peptone-agar plate colonies : Growth at 26°c. slow, circular, smooth, convex, undulate edge, greyish-white. *Beef-peptone-agar slant :* Moderate growth, convex to raised, filiform, glistening, grey, smooth, translucent, butyrous, no odour. *Gelatine colonies :* Punctiform, round to convex, undulate, smooth. *Nutrient-gelatine stab .* Slow crateriform liquefaction after 3 days. *Plain-gelatine-stab :* Slow crateriform liquefaction after 3 days. *Nutrient-broth and Tryptophane broth :* Moderate clouding in 4 days, flocculent surface growth, finely granular sediment. *Purple-milk :* Alkaline in 4 to 13 days, later acid, firm curd in 33 days, no whey. *Potato-slice :* In 9 days growth moderate, light brown, moist, smooth, potato grey. *Nitrates* Strong nitrite production in 20 days in nitrate-broth ; weak in agar media. *Indole :* Not formed. *Hydrogen sulphide :* Not formed. *Starch :* Not hydrolysed. *Ammonia :* Not formed in 20 days in broth or agar media. *Synthetic-carbohydrate media :* No gas in any medium ; acid in arabinose, glucose, fructose, sucrose, glycerol and mannitol in 14 days ; acid in lactose and maltose in 34 days but no change in 20 days and slight change in 30 days ; no acid in 34 days in Rhamnose, raffinose, melizitose, starch, inulin, dextrin, glycogen and salicin. *M.R. test.* Positive. *V. P. test* Negative

The morphological, cultural and physiological characteristics of the casual organism agree with those of *Bacterium punctulans* Bryan (1933), except that the New Zealand organism has not shown ammonia production. *Bacterium punctulans* is listed by Bergey (1939) as a probable synonym of *Phytomonas tomato* (Okabe) Magrou. More recently Dowson (1943) has classified this organism as *Pseudomonas punctulans* (Bryan) Dowson. The organism under discussion produced only a slight colour change in lactose and maltose in 30 days with a definite acid reaction in 34 days. Dowson (1939) in defining the genus *Pseudomonas* states that "none produce acid in lactose, maltose and salicin" basing his conclusions on tests which did not exceed four weeks.

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* Difco media used where applicable and methods those suggested by "Manual of methods for pure culture study of bacteria" of Society of American Bacteriologists.

CECIDOMYID MIDGES ON MEADOW FOXTAIL AND COCKSFOOT IN NEW ZEALAND

By H. JACKS and W. COTTIER, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 30th April, 1948*)

Summary

1. The midge *Stenodiplosis geniculati Reut* was reared from meadow foxtail (*Alopecurus pratensis* L.) seed-heads and *S. geniculati* Reut. var. *dactylidis* Barnes from cocksfoot (*Dactylis glomerata* L.) seed-heads collected in the provinces, Auckland, Hawke's Bay, Taranaki, Wellington, Otago and Southland. In addition the latter variety emerged from cocksfoot collected in Canterbury. No specimens of *S. geniculati* were reared from cocksfoot.

2. No specimens of *Dasyneura alopecuri* Reut. were collected during the survey and it is concluded that if this is present, it must be single-brooded as in England.

3. Colours of both sexes of *Stenodiplosis geniculati* Reut. var. *dactylidis* Barnes are described.

4. In a preliminary survey, no midges were found in seed-heads of 15 other species of Gramineae.

INTRODUCTION

DURING seasons 1937 and 1938 it was found that a Cecidomyid midge was breeding in cocksfoot (*Dactylis glomerata* L.) seed-heads in Canterbury. Specimens were sent to Dr. Barnes of Rothamsted Experimental Station, England, who stated that in his opinion the insects were a hitherto undescribed variety of *Stenodiplosis geniculati* Reut. and he described the variety under the name *dactylidis* (Barnes, 1940). The same author (Barnes, 1931) had previously stated that specimens of *S. geniculati*, reared from meadow foxtail (*Alopecurus pratensis* L.) in New Zealand were in the collection of Dr. E. P. Felt at the State Museum, Albany, N.Y., U.S.A. However, this fact was not adequately appreciated in New Zealand, it being generally considered that the Cecidomyid species on meadow foxtail here was *Dasyneura alopecuri* Reut. as reported by Cockayne (1916). In order to confirm the presence of *Stenodiplosis geniculati*, a survey on meadow foxtail was begun in 1938-39. Results of that season's survey have been reported by Barnes (1940), who identified all specimens reared as *S. geniculati*. He also mentioned he thought it likely that the species was present in New Zealand in 1916 and that possibly Cockayne (loc. cit.) was unknowingly dealing with the effects of both *S. geniculati* and *Dasyneura alopecuri*. The survey on meadow foxtail was continued in 1939-40 and extended in that season to include cocksfoot in order that the incidence of the variety *dactylidis* in the Dominion could be determined; a preliminary search for midges on other grasses was also carried out. It is the purpose of the present paper to record and discuss results obtained in 1939-40.

MEADOW FOXTAIL AND COCKSFOOT, 1939-40

Material and Methods

During this season, through the courtesy of the Fields Division of the Department of Agriculture, seed-heads of these grasses were sent to Auckland from many parts of New Zealand. Meadow foxtail was collected from November to February and cocksfoot from December to March. Altogether a total of 48 samples of meadow foxtail and 52 of cocksfoot were received. The localities in which these were taken were:

Province.	Meadow Foxtail.	Cocksfoot
Auckland	Auckland City, Dargaville, Tauranga, Te Kuiti, Warkworth, Whakatane	Auckland City, Warkworth, Whakatane
Hawke's Bay	Dannevirke, Hastings, Takapuna, Waipukurau	Dannevirke Hastings
Taranaki	New Plymouth, Stratford	New Plymouth
Wellington	Palmerston North, Wanganui.	Palmerston North, Wanganui
Canterbury		Ashburton, Christchurch
Otago	Dunedin, Ranfurly	Balclutha
Southland	Invercargill, Otatara	Invercargill

Fifty seed-heads were selected at random from each sample and each lot of 50 was placed in a separate quart glass preserving jar, the mouth of which was covered with muslin. These were kept under observation in the laboratory for 60 days. Emergence began a few days after receipt of samples and from then onwards midges were counted and removed daily.

TABLE I MEAN NUMBERS OF MIDGES EMERGED PER SEED-HEAD OF MEADOW FOXTAIL AND COCKSFOOT

Grass and Provinces	Nov.	Dec.	Jan	Feb	Mar	Totals
Meadow Foxtail						
Auckland	65.6* (7)	14.0 (3)	1.0 (3)			80.6 (13)
Hawke's Bay	16.8 (4)	12.0 (5)	-			28.8 (9)
Taranaki	0.0 (1)	3.1 (1)		-		3.1 (2)
Wellington	21.5 (4)	0.5 (1)	0.3 (2)	-		22.3 (7)
Otago	0.0 (3)	9.8 (4)	0.8 (4)			10.6 (11)
Southland	0.1 (2)	1.3 (1)	0.2 (2)	0.5 (1)		2.1 (6)
Cocksfoot						
Auckland	—	47.1 (2)	36.9 (11)	114.8 (9)	101.1 (2)	299.9 (24)
Hawke's Bay	—	71.3 (5)	0.0 (1)	—		71.3 (6)
Taranaki	—	63.7 (2)	0.7 (1)	0.0 (2)	-	64.4 (5)
Wellington	—	29.9 (2)	4.7 (3)	0.4	-	35.0 (5)
Canterbury	—	0.7 (2)	2.8 (5)	0.0 (2)	—	4.5 (9)
Otago	—	1.0 (1)	-	—	-	1.0 (1)
Southland	—	0.0 (1)	—	1.7 (1)	-	1.7 (2)

* Figures in brackets show numbers of samples received.

RESULTS

These are summarized in Table I where they are expressed as mean numbers of midges that emerged per seed-head. Localities of collection have been grouped into provinces, the figures in brackets showing the number of samples received per month from each province. A dash (—) shows that no samples from the province in question were received during the month.

DISCUSSION

It is clear that midges are distributed throughout the country, as some emerged from both meadow foxtail and cocksfoot in every locality sampled.

In the case of meadow foxtail, greatest numbers of midges per seed-head emerged from material collected in the Auckland Province. In the North Island, excluding the two Taranaki samples, midges emerged from this grass more abundantly during November than in any other month and they became progressively fewer up to February. Suitable seed-heads of meadow foxtail were becoming difficult to secure in these localities by mid-January because of dropping of seed and were unprocurable in February. In the South Island, indications were that maximum emergence was later than in the North, as this occurred in Otago and Southland in December. Total mean emergence per seed-head was also less in these provinces than in the north.

In the case of cocksfoot, emergence over the period December to March was also greatest in Auckland. In this province, however, many of the collections were made from small stands of cocksfoot about the city and may not indicate the true position in farming areas. Infestation in the North Island was considerable during December and, with the exception of Auckland, became less in the succeeding months. As with the meadow foxtail total, mean emergence of midges per seed-head was less in the South Island than in the North Island provinces.

In view of the statements of Cockayne (loc. cit.) and Miller (1918) that the midge on meadow foxtail had a serious effect on seed production, sometimes destroying as much as 70 per cent. of the crop, it would seem probable from the figures in Table I that the cocksfoot midge is equally destructive to the seed of its host.

IDENTITY OF MIDGES

All collections of midges from meadow foxtail and cocksfoot were carefully examined for identification of species. In view of the statement of Barnes (1940 and 1946) that *Stenodiplosis geniculati* occurs on cocksfoot in England and Ireland, and that he had bred the species through successfully on cocksfoot without any alteration in the characters of typical meadow foxtail specimens, it was thought that specimens of *S. geniculati* might be found among the midges from cocksfoot. Examination, however, failed to disclose this, all specimens from meadow foxtail being *S. geniculati* and all from cocksfoot *S. geniculati* var. *dactylidis*.

Dasyneura alopecuri Reut.

When this survey was originally planned it was thought that some evidence of the occurrence of *Dasyneura alopecuri* might be obtained. However, perusal of the life-cycle of this species in England shows that there it is single-brooded, with a possibility of a second brood in very favourable seasons (Barnes, 1930). The fact that no specimens of *D. alopecuri* were reared from meadow foxtail during the New Zealand survey does not mean that the species is absent from the Dominion, but it does show that if it is present as stated by Cockayne (loc. cit.) and Miller (loc. cit.), it must be single-brooded as in England.

Colours of Stenodiplosis geniculati var. dactylidis

As no previous record of the colours of this variety in life has been published, the following notes concerning them have been made.

Adult female.—Head and thorax very dusky to black. Antennae and eyes black. Halteres dusky, tinged with red. Wing insertions reddish-yellow. Wings grey, veins dusky. Legs dusky, tinged with light yellow. Abdomen brick red ornamented with dusky areas; apical two segments black.

Adult male.—Head and thorax dusky to black. Eyes black. Antennae dusky tinged with yellow. Abdomen yellow, dusky at apex. Other characters as in female.

It is interesting to note that the abdomen in the adult female is red. In his key for separation of midges attacking cocksfoot, Barnes (1940) gave the colour of the abdomen in adults of *S. geniculati* from that grass as dull honey-yellow, so that the variety *dactylidis* is further distinguished in the adult female by colour. This red colour fades rapidly after death.

OTHER GRASSES, 1939-40

In a preliminary survey for the presence of midges, seed-heads of the following grasses were collected in the neighbourhood of Owairaka, Auckland. Perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam.), prairie grass (*Bromus catharticus* Vahl.), sweet vernal (*Anthoxanthum odoratum* L.), Yorkshire fog (*Holcus lanatus* L.), *Paspalum dilatatum* Poir., tall oat grass (*Arrhenatherum elatius* (L.), Mert. et Koch), oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), tall fescue (*Festuca arundinacea* Schreb.), hair grass (*Vulpia dertonensis* (All.) Volk), red-top (*Agrostis alba* L.), Bermuda grass (*Cynodon dactylon* (L.) Pers.), Kentucky blue-grass (*Poa pratensis* L.) and Chewing's fescue (*Festuca rubra* L. var. *fallax* Hack.). The seed-heads were kept in the laboratory under the same conditions as were those of meadow foxtail and cocksfoot but no midges emerged from any of them.

ACKNOWLEDGMENT

Thanks are offered to those officers of the Fields Division, Department of Agriculture, who assisted by sending in grass samples.

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A PRELIMINARY STUDY OF THE INHERITANCE OF GRAIN WEIGHT IN WHEAT

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Summary

Inheritance of grain weight has been studied in three crosses of *Triticum durum* and one of *Triticum vulgare*. In one case there is evidence of segregation of a single recessive gene for high grain weight. In the remaining crosses, high grain weight is either completely or partially dominant, differences being determined by two or three major genes. Secondary effects of genes controlling other components of yield probably also contribute to differences in grain weight.

INTRODUCTION

THE following study of inheritance of grain weight in wheat was undertaken in an attempt to measure single gene effects and to estimate numbers of genes segregating in different crosses.

It was first necessary to study the nature of dominance deviations.† At one time it was assumed that dominance did not occur in quantitative characters but it has now been demonstrated effectively in numerous cases, though it is not always present. Sax (1923) demonstrated absence of dominance in *Phaseolus* crosses for seed size and Wexelsen (1933) obtained evidence of dominance in opposite directions in barley. We still see occasional references to the general absence of dominance in quantitative characters, e.g., Richey (1945) in developing a method for augmenting vigour in inbred lines of corn, makes the absence of dominance in quantitative characters one of his basic assumptions; he considers that genes for vigour which display dominance are distinct from genes controlling quantitative characters in general, which, according to his theory do not display dominance. Since vigour is measured quantitatively it is very doubtful whether such a distinction between the two types of gene can be drawn. The present studies being made on yield in wheat may lead to further knowledge of such dominance relationships.

Estimates of the numbers of genes controlling kernel weight in wheat have already been published by Jasnowski (1934, 1935), who obtained evidence of three gene differences between certain lines. On the other hand, Worzella (1942) states that kernal weight is multigenic. In the following experiments it appears that one to three major genes are segregating, but the possibility of secondary minor gene differences is not disproved.

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† The expression "dominance deviations" refers in this paper to deviations of the phenotypic values of heterozygotes from the arithmetic mean of the two corresponding homozygotes when gene differences are additive, and from the geometric mean when gene differences are geometric.

MATERIAL AND METHODS

Four crosses were studied. The first three were crosses between varieties of the 28 chromosome wheat, *T. durum*. Of these 150 plants of the parent lines and 400 plants of the F_2 generation were grown in one season. A sample of 100 grains from each plant was weighed, the mean weight of 1 grain being calculated in units of .001 g. The fourth cross was made between two varieties of *T. vulgare*, which has 42 chromosomes; of this were grown in one year, about 1,000 plants of each parent, 200 F_1 plants, 400 F_2 plants and over 5,000 F_3 plants. In this case all grains of each plant were counted and weighed, the mean weight of one grain being again estimated in units of .001 g.

ANALYSIS OF RESULTS

The distribution of grain weight was first plotted for a few parent lines using a class interval of 1 unit. From the standard deviation of these preliminary distributions, a corrected class interval was obtained (Sinnott and Dunn, 1932).

T. durum :

Of line E252 the standard deviation was 5.43, approximately ⅓ of which, or 2 units, was chosen as class interval. Since it was apparent that the genetic differences were fairly simple this value was later doubled to 4 units for greater ease in handling data.

T. vulgare :

In 1942 four pure lines were given a preliminary analysis :

S 1884	standard deviation	5.77	units
S 1612	,, ,,	4.58	,,
S 1556	,, ,,	3.10	,,
S 357	,, ,,	3.20	,,
	Average of standard deviations	4.16	,,

A class interval of 1.5 units was therefore chosen for use in 1942, weight having been recorded to three figures which allowed this subdivision of classes. In the following year, owing to adverse field conditions, the parents were more variable, the standard deviation of S 357 being 5.92, and that of S 1556, 3.99. The class interval was therefore increased to 2 units, which was approximately $\frac{1}{20}$ of the range of each of the above lines.

It should be stressed here that the choice of class interval is in any case rather arbitrary, and in some cases may have to exceed the statistically ideal standard of ⅓ to ¼ of the standard deviation, considerations such as ease of handling data determining a larger class interval.

The preliminary investigations of pure lines showed firstly, a general difference between the range of the two species, (Fig. 1), indicating one major type of genetic difference which we shall discuss later. Secondly, within each species there are again distinct differences in both means and variances though some lines are almost identical. Finally, within pure lines were found large seasonal differences ; Fig. 2 illustrates the difference between four plots of one line, S 357, grown in two different positions in each of two years. The major difference here is that between years and it illustrates the importance of eliminating seasonal differences from quantitative studies wherever possible.

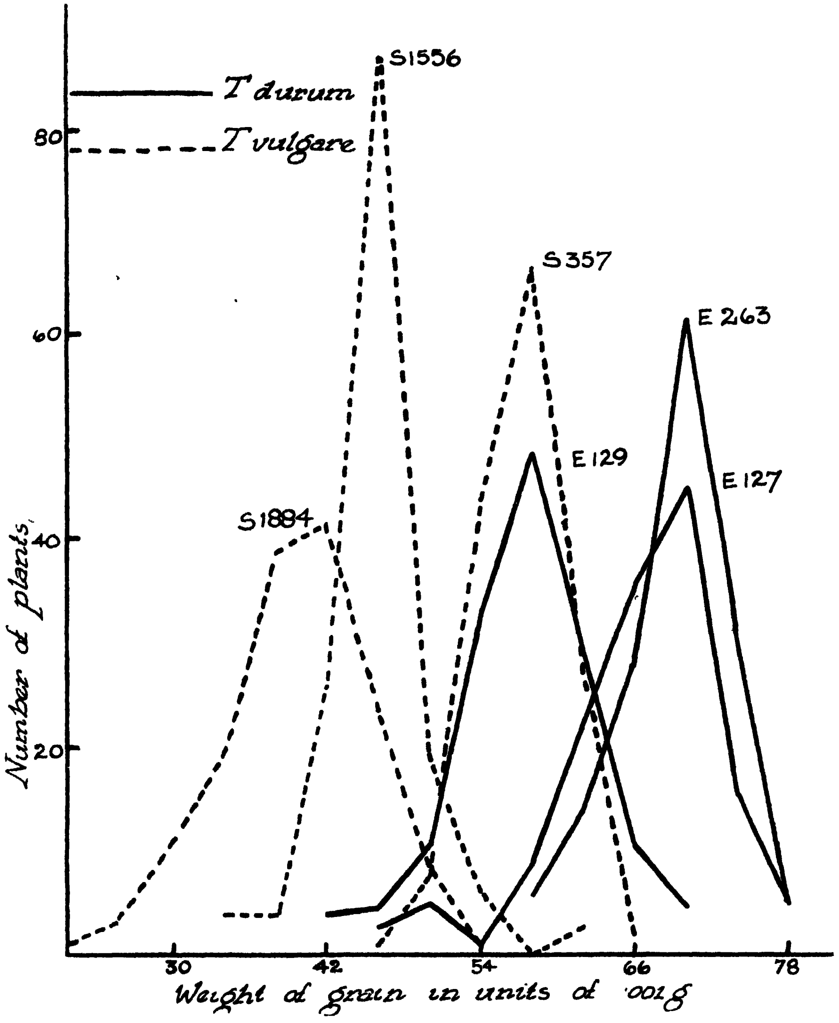

FIG. 1 Plantwise distributions of grainweight of three *T vulgare* and three *T durum* wheats.

ANALYSIS OF SEGREGATION

T. durum crosses.

The grain weights of P_1, P_2 and F_2 are shown in Table I, the lower weight parent being P_1 in each case.

TABLE I. *T. durum* CROSSES: MEANS OF P_1, P_2 AND F_2 FOR THREE CROSSES

Cross.	Grain Weight.		
	P_1	P_2	F_2
1	57.6 ± .47	66.2 ± .52	61.3 ± .42
2	58.6 ± .60	67.6 ± .46	64.9 ± .42
3	62.1 ± .35	69.4 ± .54	67.6 ± .35

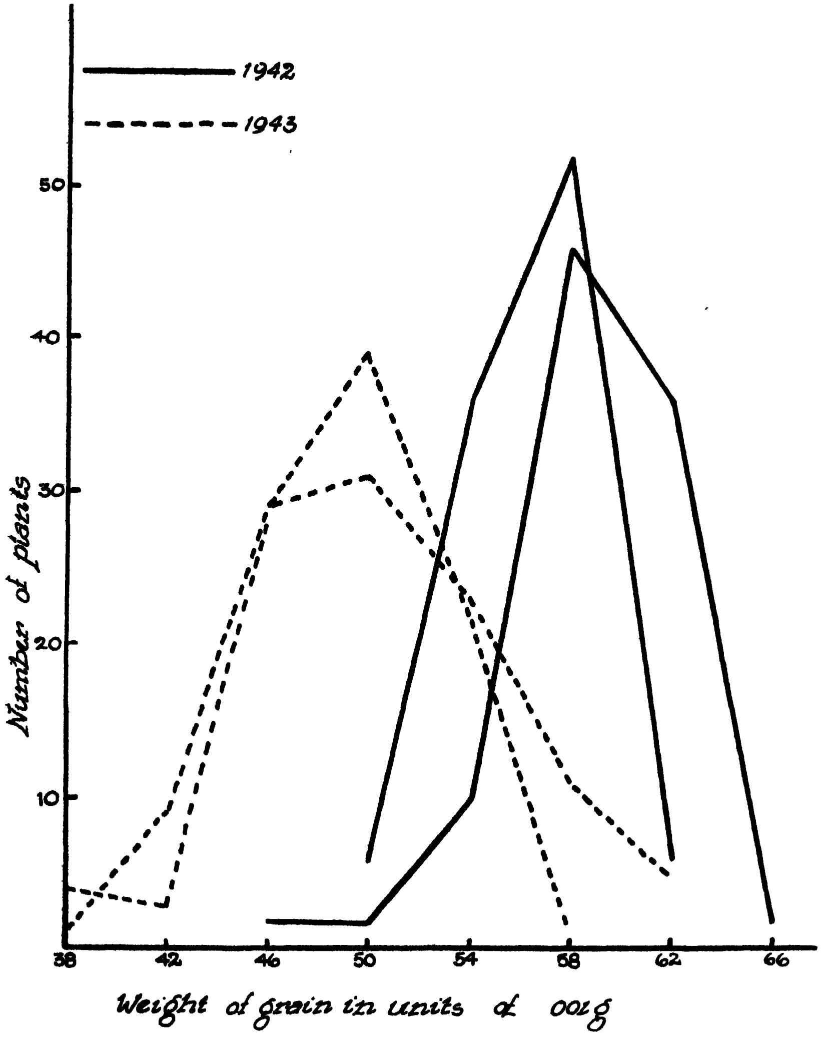

FIG. 2. -Comparison of 1942 and 1943 grain weight distributions, within and between plots

Cross 1. E129 (Valencia) × E217 (Portugal).

The F_2 mean approaches P_1 and the first assumption is made that a single gene, dominant for low grain weight, is segregating.

Assuming that a single dominant gene is segregating one would have an F_2 composed of P_1 and P_2 types in the proportion of $3P_1 : 1P_2$. Since there are 397 individuals in the F_2 population we should expect $297.75P_1 : 99.25P_2$. The $297.75P_1$ plants should be distributed about the mean (57.6) in much the same manner as in the pure line apart from a certain additional error variance which is to be expected from the mixing of P_1 and P_2 types in the F_2. Since there were 149 plants in the pure line, we may construct a theoretical distribution by multiplying each class of the actual distribution by $\frac{297.75.}{149}$ Similarly the theoretical distribution of P_2 types in the F_2 will be obtained from the actual P_2 multiplied by $\frac{99.25.}{151}$

TABLE II. COMPARISON OF ACTUAL AND THEORETICAL F_2 DISTRIBUTIONS, ASSUMING A 3 : 1 SEGREGATION (CLASS 46 INCLUDES ALL VALUES BELOW AND UP TO 46 ; CLASS 74 INCLUDES ALL VALUES FROM 74 UPWARDS)

Class value. Distribution of :	46	50	54	58	62	66	70	74	T
Actual $P_1 \times \frac{297.75}{149}$	17.98	21.98	65.94	97.92	61.95	21.98	9.99		297.74
Actual $P_2 \times \frac{99.25}{151}$	1.97	3.29	.66	5.92	19.72	23.66	30.24	13.81	99.27
Theoretical F_2	19.95	25.27	66.60	103.84	81.67	45.64	40.23	13.81	397.01
Actual F_2	20	19	45	94	86	56	44	33	397
Difference	.05	6.27	21.60	9.84	4.33	10.36	3.77	19.19	.01
x^2	.00	1.56	7.01	.93	.23	2.35	.35	26.67	39.10 $P<.01$

The theoretical F_2, obtained by assuming these two distributions, is shown in the third line of Table II. The next line of this table gives the actual F_2 distribution and below this is shown the x^2 value for each class. The total x^2 value is 39.10 which indicates more than random difference between the two populations. The source of this deviation is shown quite well in Fig. 3a, where the actual F_2 curve lies above the theoretical curve throughout the whole range. This can be corrected by postulating partial, but almost complete dominance so that instead of $3P_1 : 1P_2$ we have $1P_1 : 2$"F_1" $: 1P_2$. Now there is no F_1 curve for comparison but a hypothetical curve has been constructed by increasing the mean of P_1 to 59.6 (Table III). The result is shown in Fig. 3b, x_2 is now not significant, and the two curves agree very closely.

TABLE III. COMPARISON OF ACTUAL AND THEORETICAL F_2 DISTRIBUTIONS, ASSUMING A 1 : 2 : 1 SEGREGATION, THE F_1 SHOWING PARTIAL DOMINANCE (CLASS 46 INCLUDES ALL VALUES BELOW AND UP TO 46 ; CLASS 74 INCLUDES ALL VALUES FROM 74 UPWARDS)

Class value, Distribution of .	46	50	54	58	62	66	70	74	T
Actual $P_1 \cdot \frac{99.25}{149}$	5.99	7.33	21.98	32.64	20.65	7.33	3.33		99.25
Actual $P_1 \times \frac{198.50^*}{149}$	8.65	10.66	29.31	54.62	53.29	27.98	10.66	3.33	198.50
Actual P_2 $\frac{99.27}{151}$	1.97	3.29	.66	5.92	19.72	23.66	30.24	13.81	99.27
Theoretical F_2	16.61	21.28	51.95	93.18	93.66	58.97	44.23	17.14	397.02
Actual F_2	20	19	45	94	86	56	44	33	397
Difference	3.39	2.28	6.95	.82	7.66	2.97	.23	15.86	.02
x^2	.69	.24	.93	.01	.63	.15	.00	14.68	17.33 P .015

* Whole P_1 distribution has been moved up half a class interval thus increasing the mean of the theoretical F_1 to 59.6 as compared with 57.5 in the previous case.

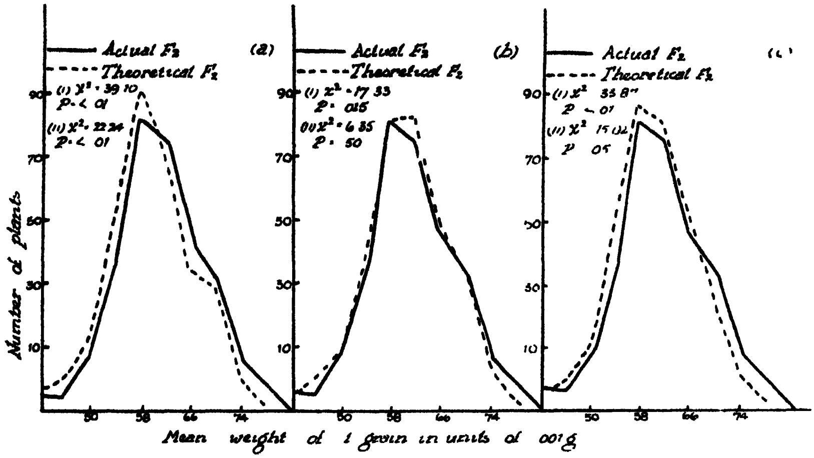

FIG 3 -Comparison of theoretical and actual F_2 distributions *T durum* cross 1

(a) $3P_1$ $1P_2$ (b) $1P_1$ $2F_1$ $1P_2$

(c) $9P_1$ 6 intermediate $1P_2$

(i) x^2 calculated over whole range

(ii) x^2 calculated from 46 to 74 inclusive

It will be asked, would not equally close agreement be obtained by constructing theoretical curves for two or more genes ? For two genes, assuming that the parent types are the extremes we have $9P_1$ 6 Intermediate : $1P_2$ (Fig. 3c). The intermediate is necessarily quite hypothetical and has been obtained by moving the P_1 distribution up to an intermediate mean value Agreement as shown in Fig 5 is not as good as for the simpler assumption, which can be accepted in so far as it is not disproved.

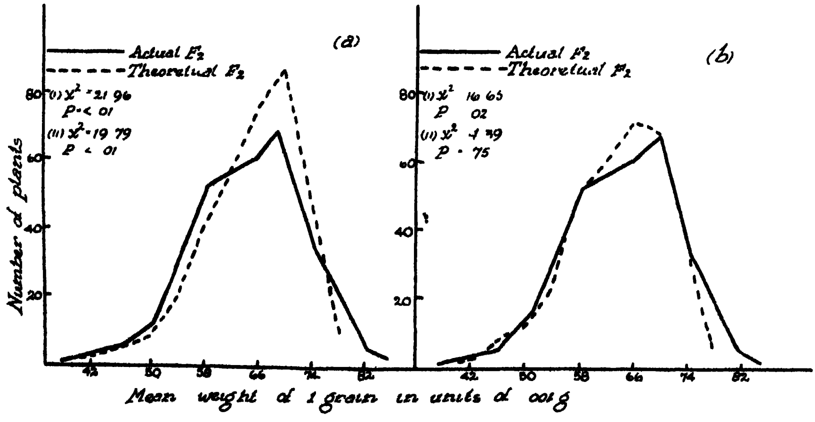

FIG. 4 -Comparison of theoretical and actual F_2 distributions, *T. durum* cross 2

(a) $1P_1$ $3P_2$. (b) $7P_1$ · $9P_2$.

(i) x^2 calculated over whole range.

(ii) x^2 calculated from 46 to 74 inclusive

Cross 2. E156 (Portugal) × E252 (Morocco).

Since the F_2 mean approaches P_2 the first assumption is made that a single gene dominant for large grain weight is segregating. Fig. 4a shows quite clearly that a 1 : 3 segregation is not a satisfactory assumption x_2 having a highly significant value. The error appears to be due to exaggeration of the frequency of P_2. An assumption of an intermediate F_1 value does not appear to be reasonable, since the actual F_2 tends to be bimodal, indicating a possibility that there are two classes. Such a curve would arise if two complementary genes were segregating, giving a ratio of $7P_1$: $9P_2$. This distribution has therefore been tested and gives good agreement between theoretical and actual values (Fig. 4b).

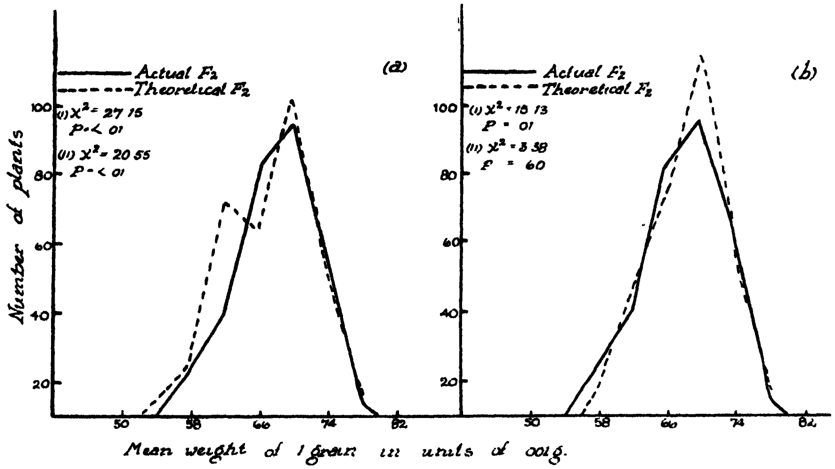

FIG. 5.—Comparison of theoretical and actual F_2 distributions, *T. durum* cross 3.

(a) $1P_1$: $3P_2$. (b) $1P_1$: 6"F_1" : $9P_2$.

(i) x^2 calculated over whole range.

(ii) x^2 calculated from 58 to 78 inclusive.

Cross 3. E178 (Portugal) × E263 (Morocco).

This again gives poor agreement for a 1 : 3 segregation (Fig. 5a), and as it does not show an obvious bimodal distribution, a 1 : 6 : 9 segregation for two genes has been tested (Fig. 5b), with quite good results. Various assumptions were tested for this cross, none being more satisfactory than the 1 : 6 : 9 segregation which is tentatively accepted.

To summarize the 28 chromosome *T. durum* crosses then, we find that in one cross low grain weight was partially dominant, the parents differing by one gene.

In the second cross the parents differed by two complementary genes dominant for high grain weight and in the third, high grain weight is again dominant, the number of genes being indefinite, but possibly equal to two.

T. vulgare cross. S357 (Crete) × S1556 (India).

T. vulgare wheats, as was shown in Fig. 1, tend to have lower grain weights than varieties of *T. durum* even though, according to mathematical expectations the larger number of chromosomes might be expected to carry a greater number of genes. This specific decrease in size is probably due to a complete alteration in the balance and interaction between chromosomes and it would in fact be a waste of time to attempt to interpret such a size difference in terms of number of genes. Within the species however, it is reasonable to attempt an analysis of the number of genes segregating.

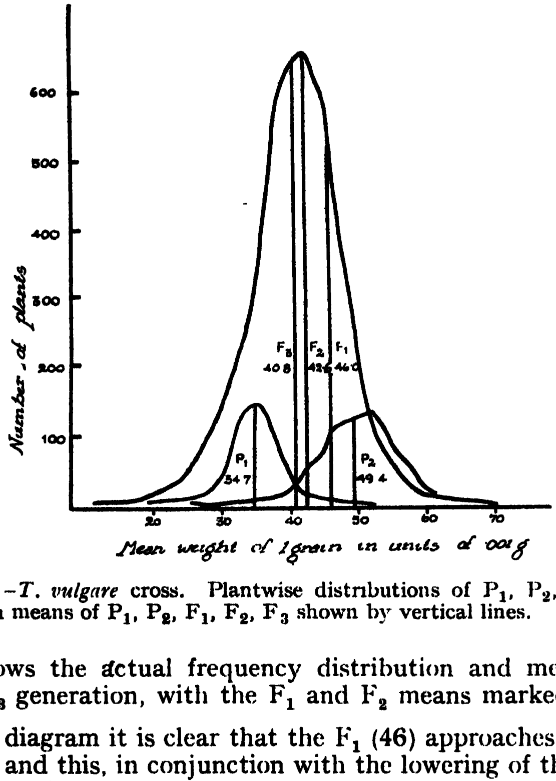

FIG. 6. –*T. vulgare* cross. Plantwise distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 shown by vertical lines.

Fig. 6 shows the actual frequency distribution and means of P_1, P_2 and the F_3 generation, with the F_1 and F_2 means marked.

From this diagram it is clear that the F_1 (46) approaches the higher parent (49.4), and this, in conjunction with the lowering of the F_2 mean to 42.6 and the F_3 to 40.8 indicates that the balance of dominance is for high grain weight. The dominance is not complete however, and the intermediate nature of the F_1 shows that either all genes segregating are only partially dominant, or that some genes are dominant for high grain weight and a fewer number are either dominant for low grain weight or are non-dominant. Both situations may of course occur simultaneously.

Considering the F_2 and F_3 distributions the relative frequency of the plants is too low for the hybrid distribution to be explained on a single gene basis, but a two gene segregation appears to be fairly reasonable. To compare the two assumptions of partial dominance and incomplete dominance, the following theoretical distributions have been constructed (Fig. 7).

1. Two genes segregating—both partially dominant, the theoretical F_1 coinciding with the actual F_1.

2. Three genes segregating—two completely dominant for high grain weight, and one completely dominant for low grain weight the theoretical F_1 coinciding with the actual F_1.

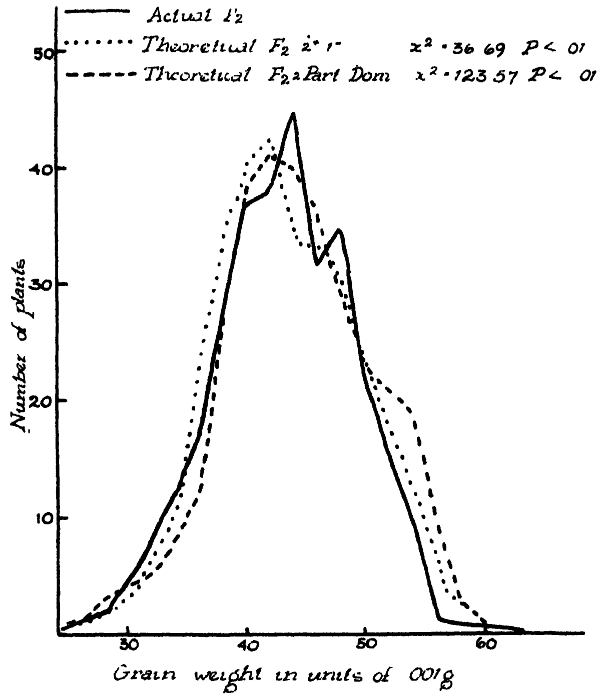

FIG. 7. - *T. vulgare* cross. Comparison of actual F_2 distribution with two theoretical F_2 distributions.

Both these assumptions give quite good agreement in the F_2, the first being rather more satisfactory than the second. In the F_3 however, agreement is not very good (Fig. 8a), although it is very much better than that expected for a five gene segregation (Fig. 8b).

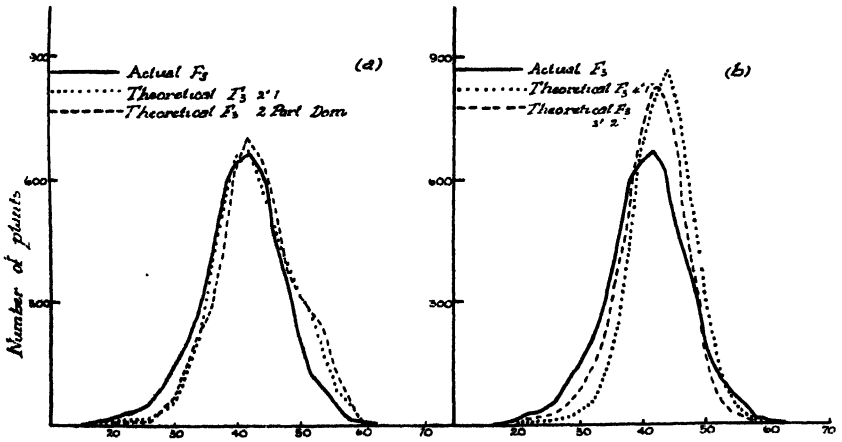

FIG. 8.—*T. vulgare* cross. Comparison of actual F_3 distribution with theoretical distributions for (a) two or three gene segregations, (b) five gene segregations.

Increasing numbers of genes were tested for each of the two main assumptions, but they gave increasingly poor comparisons with the actual data. Fig. 8b for example shows the expected F_3 distribution for five genes, four dominant + and one dominant —, the F_1 coinciding with the actual F_1, and with geometrically increasing class intervals ; and also for five genes three dominant + and two dominant —.

It should be emphasized here that the apparent one, two or three gene segregations may result from the segregation of tightly linked gene complexes, and a further programme of repeated crossing might result in the breakdown of these complexes (Mather, 1943).

DISCUSSION

The experiments described in this paper were of an exploratory nature and definite conclusions cannot be drawn. There are clear indications, from the nature of the F_2 distributions, that a few major genes are segregating, determining the general shape of the F_2 curves. However, agreement of theoretical and actual curves is not perfect, and the discrepancies may be due to the segregation of one to many minor genes. In addition, in each cross discussed above, where the segregation of more than one gene was postulated, equal effects were assumed for all the genes. This gave sufficiently good agreement for the general shape of the F_2 and F_3 curves ; but, apart from the polyploid nature of *Triticum*, there is no evidence in support of the assumption of equal gene differences (Pease, 1940). Since no more than three genes have been postulated, it is possible that these may occur on chromosomes of common origin, but even so, they may no longer exhibit equal effects. Only a long term selection programme can decide the relative importance of the size differences due to major and minor genes in this material. Minor fluctuations are likely, of course, to result from secondary effects of major gene differences affecting the development of other components of yield ; it is hoped that a more detailed study, now under way, of all yield components, will reveal the extent and nature of such apparent " minor " gene differences.

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A NOTE ON HETEROSIS IN A *TRITICUM VULGARE* CROSS

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(*Received for publication, 1st September, 1947*)

L. Powers (1944) suggests an expansion of Jones' theory for the explanation of heterosis. In studying certain maize crosses, Powers separates yield into its components and shows that heterosis in total yield depends on the multiplication together of these components which, individually may not display any degree of heterosis. A similar situation which is at present being studied in wheat is briefly reported here.

Total yield per plant (Y) in wheat is determined by the product of number of ears per plant (e), number of grains per ear (n) and mean weight of one grain (g). In one cross it was found that P_1 carried genes for high n, and P_2 genes for high e and high g, the F_1 combining all these desirable characters to give positive heterosis in Y. From Table I it can be seen that e is the only component of yield which displays heterosis, high n and high g being only partially dominant. Since, however, the F_1 value for e is only 12.6 per cent. higher than P_2, while the F_1 for Y is 32.7 per cent. higher than P_2, it is evident that the remaining 20.1 per cent. increase in yield is due to ng. That is, the multiplication of e by two partially dominant components of yield has greatly increased the hybrid vigour of the F_1. Figs. 1, 2, 3 and 4 illustrate the heterosis of e and Y and the partial dominance of n and g. In each case the drop in mean from F_1 to F_2 and to F_3 is typical of genes dominant for high yield. The extremely skew distribution of the F_3 for Y (Fig. 4) is due to the positive correlation of yield components and must not be confused with skewness due to dominance of genes for low yield.

TABLE I MEAN YIELD AND ITS COMPONENTS IN P_1, P_2 AND HYBRID GENERATIONS

Generation.	Yield Component.				Yield
	e	n	g(.001g)	ng(g)	Y(g.)
P_1	4.80 ± .08	26.6 ± .27	34.7 ± .16	.923	3.86 ± .10
P_2	5.47 ± .08	18.7 ± .16	49.4 ± .20	.924	5.10 ± .10
F_1	6.16 ± .17	25.0 ± .44	46.0 ± .44	1.150	6.77 ± .27
F_2	5.21 ± .11	21.3 ± .40	42.6 ± .39		4.55 ± .15
F_3	4.88 ± .03	20.5 ± .09	40.8 ± .10		3.91 ± .03

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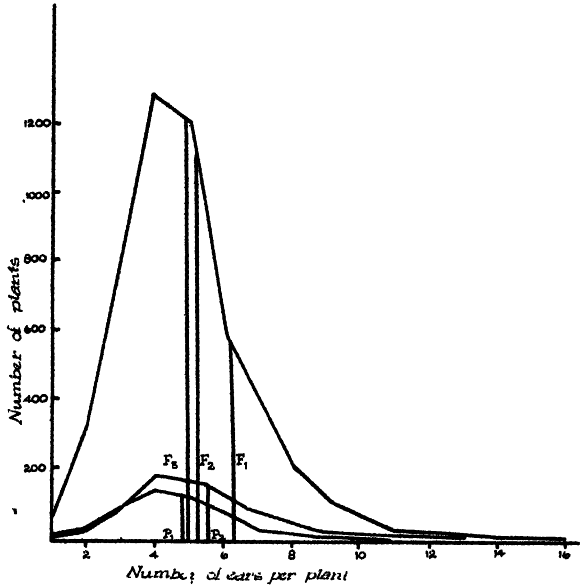

FIG. 1.—Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for number of ears per plant.

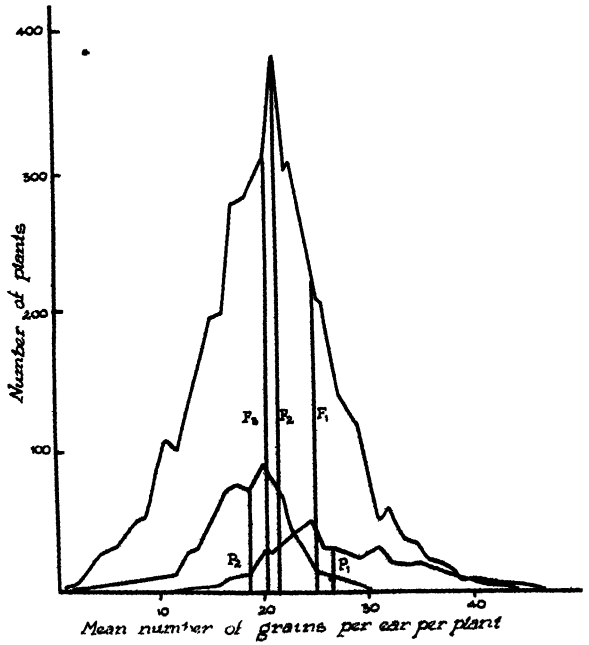

FIG. 2.—Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for mean number of grains per ear per plant.

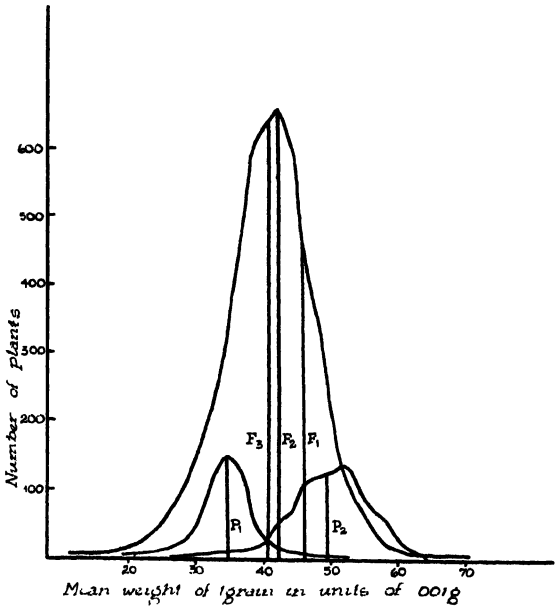

FIG. 3. -Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for mean weight of one grain, per plant.

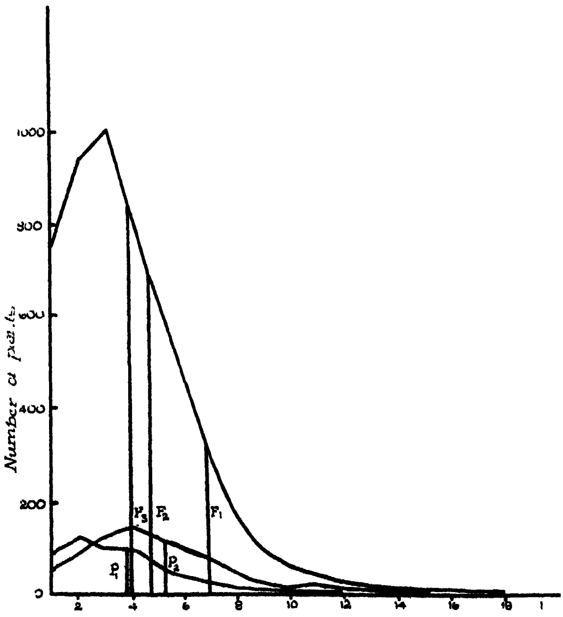

FIG. 4.—Frequency distributions of P_1, P_2, F_3 with means of P_1, P_2, F_1, F_2, F_3 for yield per plant.

COBALT, COPPER AND IRON IN THE LIVER IN RELATION TO COBALT DEFICIENCY AILMENT.

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Summary

Good correlation has been shown between both cobalt concentration and total cobalt in the liver and incidence of bush-sickness in the North Island Evidence is submitted that the correlation is with cobalt deficiency ailment and not with unthriftiness Some low figures have been found for healthy animals from marginal deficiency areas

The copper content of the liver in bush-sickness is much higher than in enzootic marasmus and coast disease No gross accumulation of iron in the liver is observed in bush-sickness.

The reference data reported have been successfully used for several years for chemical diagnosis of cobalt deficiency Reference criteria for the interpretation of the analytical results are given

INTRODUCTION

EARLY analyses of livers from healthy and bush-sick North Island sheep (1, 2, 3) indicated some correlation between cobalt content and incidence of bush-sickness. As the information from this and other related work (4) was not sufficient for diagnostic purposes, a more extensive survey was undertaken commencing early in 1938. War delayed the completion of this work, the combined results of which are presented in this paper. In the intervening period further results for cobalt in livers of sheep have been published (5, 6, 7, 8, 9, 10), and these are in general agreement with the values here reported.

Owing to the fact that "coast disease" in South Australia was shown to be a combined cobalt and copper deficiency ailment (11, 12), while both this disease and enzootic marasmus in Western Australia were shown to be accompanied by abnormal accumulation of iron in the liver (12, 13), copper and iron were also determined on most samples. A detailed investigation of the copper status of sheep and cattle in New Zealand has since been made by Cunningham (14).

MATERIALS ANALYSED

Liver samples analysed were from the following groups :

(a) Healthy animals from districts not affected by typical bush-sickness. Through the co-operation of the District Superintendents of the Livestock Division of the Department of Agriculture, and the local meat and stock inspectors, samples from animals of known history were obtained.

(b) Healthy treated animals from typical "bush-sick" areas (mostly from Mamaku).

(c) Known "bush-sick" animals, that is, animals which had been grazing on a known deficiency area and which showed symptoms consistent with cobalt deficiency.

(d) Animals suffering from ailments other than cobalt deficiency (i) facial eczema (ii) enzootic icterus or toxaemic jaundice (iii) fed toxic amounts of copper (iv) copper deficiency (v) fed excessive doses of cobalt.

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Though the most susceptible animals are lambs of 4 to 9 months and calves or yearlings of 6 to 18 months, older animals, especially among sheep, will succumb to bush-sickness if grazed long enough on severely deficient pastures without cobalt supplements. Lambs younger than 4 months and calves younger than 6 months do not usually show gross symptoms of bush-sickness, but the progeny of bush-sick ewes and cows seldom thrive, even at such early ages (9). As livers from animals of widely varying ages are frequently submitted for determination of cobalt status it was considered desirable to have reference data, where possible, for animals of all ages from foetal stage to maturity.

CHEMICAL METHODS

REMOVAL OF ORGANIC MATTER

For cobalt determinations in the later work the wet digestion technique (3) was replaced by the following nitric acid ashing procedure, because of the difficulty experienced in fuming off the residual sulphuric acid conveniently and without losses by creep.

A 10g. dry weight of sample, or about 30g. wet tissue, is warmed on the hot plate in a fume cupboard in a 4 in. silica basin with about 25 ml. 50 per cent. nitric acid (equal volumes concentrated nitric acid and water), till frothing commences. The basin is removed from the hot plate and 5 ml. amyl alcohol added to suppress the frothing. Part of the alcohol reacts with the nitric acid and part boils off. When the reaction ceases, heating is continued till charring. The charred residue is ashed in the fume cupboard at 550 to 600° c. for about 30 minutes. The basin is cooled, about 15 ml. 50 per cent. nitric acid added, and, with a clock-glass on the basin to produce reflux, heating is continued till all the acid has been driven off. The residue is again ashed for 5 minutes, preferably at a lower temperature, about 500 c., to avoid damage to the basin by fusion of the ash The nitric acid treatment followed by ashing is repeated till a residue completely free from carbon is obtained.

The above method is suitable for approximate determinations of copper on the same sample but appears to be unsuitable for the simultaneous determination of iron, for which a separate digest is necessary. The wet digestion procedure (3) has the advantage that it allows of reasonably accurate copper and iron determinations on the same digests.

PREPARATION OF EXTRACTS FOR COBALT DETERMINATION

The subsequent treatment of the ash is similar to that previously outlined (3) except that the final evaporation to dryness is carried out at a higher temperature, for example on the sandbath, to ensure that no traces of aqua regia remain. If a permanganate-like colour develops, re-evaporation to dryness with hydrochloric acid is used.

METHOD OF ESTIMATION

Cobalt

All analyses prior to December, 1936, including the results reported by Josland (15, 16), were obtained by a modification of the method of Stare and Elvehjem (17) in which neutralization of the test solution is carried out with caustic potash in the presence of the nitroso-R-salt reagent. From that time till November, 1939, the solutions were neutralized first before addition of the reagent (3), a procedure which was subsequently shown to give low results for some solutions (18).

From November, 1939, a reliable technique eliminating the use of caustic alkali has been used (19) and all the results here reported, unless otherwise indicated, have been obtained by this procedure.

Copper

The diethyldithiocarbamate reagent has been used throughout. Both the amyl alcohol extraction method (20) and Hoar's method of stabilizing the colour with gum arabic (21) have been used, the latter especially for amounts of copper in excess of 100 p.p.m.

Iron

Determinations of iron were made at the same time, using a, a^1-dipyridyl. As this reagent gives some colour with copper as well as with ferrous iron, appropriate corrections were made by using a separate tintometer graph. No great accuracy can be claimed for such a method (error about ± 10 per cent.) but tests gave good agreement with results by the thiocyanate method, also affected by copper, using as standards iron solutions containing comparable amounts of copper.

RESULTS

The results for both concentration and total amounts of cobalt, copper and iron in the livers, are summarized in Tables I to IV. Only results for livers from animals of known state of health are shown. Diagnostic material, from unthrifty animals affected by some unknown complaint not of parasitic origin, or samples submitted for cobalt status, are not included in the tables. Concentrations are reported as parts of the element per million (p.p.m.) of dry matter (constant weight at 105° C.), and total amounts are expressed in milligrams of the element. Where there are only two samples in the particular group, the cobalt, copper and iron figures are kept in correct relative order.

The effect of age of the animals on size of the liver makes it necessary for an examination of total cobalt levels to separate the animals into different age groups. For sheep, 3-9 months, 12-20 months, and 24 months and older were selected as these are the normal age groups at slaughter. For cattle the ages are less clearly defined, namely " yearlings " 6 months to 2 years, and " mature " beasts more than 2 years (usually more than 3 years).

DISCUSSION

COBALT IN LIVERS OF HEALTHY ANIMALS

As a basis for reference and discussion, values for normal healthy animals are considered first. Table V shows results for animals other than sheep and cattle.

(1) *Effect of age of animals on cobalt content of the liver.*

(a) *Sheep.*

In healthy sheep on a steady, natural, unsupplemented, cobalt diet some increase in concentration of cobalt from birth through to weaning is indicated from the results in Table I. The figures for two of the new-born lambs and the 2, 4 and 8 weeks lambs as well as 2 figures each for 3, 5 and 6 months lambs form a truly comparable series. These were from a uniform group of lambs born and raised on a paddock on the Horotiu-Te Kowhai formation at the Ruakura Animal Research Station, commencing from mid-August, 1939. These animals received no cobalt supplements of any kind, while the evidence from analysis of pastures in the previous season is that the cobalt content of the pasture would be fairly constant (see reference (22) Table II, p. 97B), though slightly higher at the time the new-born and six months lambs were killed. No highly significant differences in cobalt concentration were found comparing the new-born, two weeks, and four weeks lambs with three months lambs, but compared with six months lambs the differences were statistically significant at the 1 per cent. level.

When the figures in Table I for animals 3 months and older are further analysed, we find in the 3-9 months age group 59 samples averaging 0.159 p.p.m. (range 0.063 to 0.322); 12-20 months 5 samples averaging 0.155 p.p.m. (range 0.111-0.190); 2 years and older 6 samples averaging 0.252 p.p.m. (range 0.183-0.315). Of the 59 samples from healthy 3-9 months lambs, only 5, all less than 7 months old, showed figures of less than 0.10 p.p.m. cobalt. Although the number of samples in the oldest age group is small, the average cobalt level is appreciably higher than in the 3-9 months age group.

In healthy 3-9 months lambs total cobalt ranged from 0.010 to 0.050 mg., average 0.023 mg., in 12-20 months sheep 0.019 to 0.025, average 0.023 mg.; and for sheep 2 years and older 0.027 to 0.073, average 0.051 mg.

(b) *Cattle.*

In new-born and bobby calves the cobalt concentration is significantly lower than in healthy yearling and mature cattle beasts (Table I).

In new-born and bobby calves, total cobalt varied from 0.004 to 0.035 mg., average 0.014, while in six healthy yearlings the range was 0.129 to 0.168 mg., average 0.146.

(2) *Effect of cobalt level in the diet on cobalt content of the livers of healthy animals.*

(a) *No cobalt supplements.*

In healthy sheep in "healthy" districts, the few examples where relevant data on cobalt content of the pasture are available, indicate some correlation between cobalt concentration in the liver and dietary cobalt, especially where differences in dietary levels are pronounced. For sheep kept healthy in bush-sick country by means of cobalt supplements, see Table VI and subsequent discussion. The lowest of twelve results for lambs from the Wairarapa district, namely 0.063 p.p.m. (0.013 mg. total cobalt) was for an animal from Mt. Bruce district where Maunsell and Lamont (23) found low levels of cobalt in some pastures (0.04 to 0.06 p.p.m.), while the highest value, 0.26 p.p.m. (0.024 mg. total cobalt), was for a lamb from an area associated with relatively high cobalt levels in the pastures (approx. 0.25 p.p.m.). On the other hand a comparison of two groups of 5-6 months lambs from Ruakura, one grazing on a pasture which provided on a yearly average approximately 0.13 p.p.m. cobalt (paddock 13A, reference (22)), the other 0.20 p.p.m. cobalt, showed no statistically significant differences in concentration, though seven of the eight values showed consistent correlation.

(b) *Cobalt supplements.*

In healthy districts, animals which have had access to proprietary licks containing small amounts of cobalt gave an average cobalt concentration of 0.22 p.p.m. as compared with 0.17 for those receiving no supplements. Where drenches containing very small amounts of cobalt have been used, the average figure is 0.18. In neither case is the difference significant, probably because the amount of cobalt used in such proprietary licks and drenches in healthy areas was initially very small. *Note:* Cobalt licks, used in the main deficiency areas contain higher levels of cobalt, namely the recommended amount of 4 oz. cobalt sulphate per ton (24), and can be expected to give greater differences.

Josland's work (16) has shown that much larger doses of cobalt result in greatly increased cobalt levels in the liver. With injurious amounts of cobalt, figures in excess of 3 p.p.m. cobalt have been obtained.

COBALT IN LIVERS OF BUSH-SICK ANIMALS

(Results are summarized in Table III.)

(1) *Effect of age of animals on cobalt content of the liver.*

Sheep.

The livers from the two foetal and new-born lambs from bush-sick ewes show concentrations of cobalt comparable with those in 3 months and older sheep affected by bush-sickness. Data from other animals of less certain history and not reported in the table confirm this trend. This means a progressive increase in total cobalt content roughly proportional to the weights of the livers. Five lambs 5-9 months old gave an average figure of 0.039 p.p.m. (range 0.029-0.045), 15 sheep 12-20 months 0.024 p.p.m. (range 0.008-0.040) and 10 sheep 2 years and older 0.041 p.p.m. (range 0.033 to 0.054).

Cattle.

No information is available on the effect of age on the cobalt content of livers of bush-sick cattle beasts.

(2) *Effect of cobalt level in the diet on cobalt content of the liver.*

The improvement in health of sheep and cattle on North Island bush-sick pastures from the use of cobalt supplements in the form of licks, drenches or topdressing has been referred to in various publications (24) to (32). The results of the extensive series of topdressing experiments instituted at Mamaku in 1938, show good correlations between cobalt content of the pasture and cobalt content of the liver, and both with incidence of deficiency symptoms (Table VI).

Twenty-three of the thirty samples from bush-sick animals reported in Table III were from these and earlier Mamaku experiments and all without exception were associated with pastures whose average cobalt content for monthly samples taken over the preceding 12 months did not exceed 0.06 p.p.m. cobalt (range 0.04 to 0.07, average 0.055 to 0.061). This means that figures of 0.008 to 0.054 p.p.m. cobalt, average 0.031 p.p.m., in the liver have been associated with an average yearly cobalt content of the pasture of 0.06 p.p.m. On the cobalt topdressed pastures, however, breeding ewes and lambs have been maintained in excellent condition. In these the cobalt content of the livers is relatively high, in accord with the increased cobalt content of the pasture.

DIAGNOSTIC VALUE OF COBALT CONTENT OF LIVERS

(1) *Correlations.*

The results in Tables I to III and VI show a fair correlation between both cobalt concentration and total cobalt in the liver and incidence of bush-sickness. The results of analyses of livers from animals suffering from other known ailments (Table IV) and also the unreported results of analysis of diagnostic samples from unthrifty animals from " healthy " districts, which have shown quite high cobalt levels, confirm that the correlation is with cobalt deficiency and not with unthriftiness.

(a) *Animals at an age when they are particularly susceptible to bush-sickness.*

Sheep.

The livers of typical North Island bush-sick sheep 5 months and older contained 0.03 p.p.m. cobalt on dry matter (range 0.01 to 0.05), while normal healthy untreated sheep 3 months and older from healthy districts averaged 0.17 p.p.m. cobalt (range 0.06 to 0.32, most values being above 0.10 p.p.m.). When the figures for sheep 2 years and older are examined, we find a still more pronounced difference in cobalt concentration (average for 10 bush-sick ewes 0.041 p.p.m., range 0.033 to 0.054) as against 0.252 p.p.m. (range 0.183 to 0.315) for healthy ewes. Sheep kept healthy in bush-sick country by means of adequate cobalt supplements have shown still higher average levels (0.36 p.p.m.) though the values are more variable (range 0.08 to 1.2).

Similar or slightly lower cobalt levels in the livers of sheep affected by cobalt deficiency ailment similar to bush-sickness were obtained by South Island workers at Glenhope, Nelson (4, 6, 10) and at Morton Mains, Southland (4, 7, 8).

Total cobalt in the livers of typical bush-sick sheep 5-9 months old ranged from 0.001 to 0.008 mg. (average 0.004) as compared with 0.010 to 0.050 (average 0.023) for healthy 3-9 months sheep. For 12-20 months the figures were respectively 0.002 to 0.007 (average 0.004) and 0.019 to 0.025 (average 0.023), and for 24 months and older 0.004 to 0.012 (average 0.006) and 0.027 to 0.073 (average 0.051).

Cattle.

For bush-sick yearling heifers and steers the values for concentration of cobalt were 0.03 p.p.m. (range 0.02 to 0.04) and for healthy yearling or mature cattle beasts 0.24 p.p.m. (range 0.12 to 0.40).

Total cobalt in the liver of the one bush-sick yearling heifer for which total weight of the liver is available gave 0.04 mg. Co., as compared with an average of 0.15 mg. (range 0.13 to 0.17) for six healthy yearlings from healthy districts.

(b) *Animals which normally do not show symptoms of bush-sickness.*

Sheep.

From the very limited data available, supplemented by data not here tabulated from animals of less certain history, it appears that the cobalt concentration in the livers of foetal and new-born lambs from bush-sick ewes is similar to that of sheep 3 months and older. On the other hand, foetal and new-born lambs from healthy ewes in healthy districts (Table I) show an average cobalt level of 0.08 p.p.m. (range 0.05 to 0.12). New-born lambs from ewes kept healthy in bush-sick country by means of cobalt supplements show an average cobalt level in the liver of 0.15 p.p.m. (range 0.11 to 0.20).

Cattle.

No data are available for comparison of levels in new-born calves from bush-sick cows with those from healthy mothers.

Other animals.

In New Zealand cobalt deficiency is known only in sheep and cattle. Analyses of two samples from a case of suspected deficiency in goats on a farm at Mohaka on East Coast hill country associated with cobalt deficiency in sheep gave inconclusive results (Table V). Cunningham in 1937 (33) and Underwood and Elvehjem (34) were unable to produce cobalt deficiency in rats, possibly because in both cases the purified diets still contained cobalt (34, 35).

Anomalous results.

Where cobalt drenches have been given to bush-sick animals *in extremis* in an unsuccessful attempt to save their lives, the cobalt level in the liver may increase to normal levels. Samples from such animals or from any cobalt treated animals are usually unsuitable for diagnostic purposes.

The only serious anomalies in the reference data were for two livers from sheep from Okaihau, North Auckland (Table I, A (2)). Total cobalt could not be determined. Two samples from Taranaki which gave rather low results of 0.050 and 0.056 p.p.m., the latter 0.008 mg. total cobalt, have also been included in this section. Although these four animals were described as healthy, they were all from farms with histories of ailment consistent with mild cobalt deficiency.

Askew, in experiments at Glenhope in the South Island (6), concluded that a cobalt content of 0.05 p.p.m. on dry basis in the livers of 2½ to 3 year old sheep and 0.010 mg. cobalt per organ, is compatible with perfect health. Later Askew and Watson (10) demonstrated that the cobalt level in the liver drops to quite low levels before the development of typical deficiency symptoms. Furthermore, cobalt contents as low as 0.04 p.p.m. had been reported for healthy animals at Morton Mains in Southland (4). The conclusion from the present work, however, is that such low levels of cobalt, though sometimes associated with healthy animals, are indicative of sub-optimal or dangerously low amounts of cobalt in the diet. In diagnostic work, therefore, such figures are treated as indicative of deficiency of cobalt.

APPLICATION OF RESULTS

(1) *Cobalt concentrations.*

From an early stage in this investigation, tentative diagnoses were made with the following results:

As the highest cobalt figure yet noted in this work for the liver of a healthy sheep which had received no cobalt supplements is 0.32 p.p.m. and for cattle, 0.40 p.p.m., figures above 1 p.p.m. cobalt have been regarded as indicating abnormally high cobalt in the diet through access to cobalt other than that present in the untreated pasture.

Sheep.

Fifty-three cases in animals 4 months or older, which showed a range of cobalt concentrations from 0.015 to 0.060 p.p.m. were tentatively diagnosed as cobalt deficient. Of these, 38, including that associated with the 0.060 p.p.m. figure in a 6 months lamb, have been sustained by subsequent good results with other sheep from the use of cobalt supplements. In the remaining cases, information has been insufficient to justify any conclusion. In 6 out of 8 cases, tentatively classed as "border-line" (animals 6 months or older, range of cobalt concentrations 0.063 to 0.091 p.p.m.) there is evidence of subsequent benefit to other sheep in the flocks from the use of cobalt. In one instance, two apparently similar lambs from the one draft gave values of 0.047 and 0.087 p.p.m. cobalt respectively. A controlled drenching experiment proved clearly the benefit of cobalt on the farm concerned. This variability of results due to " sampling error " shows that isolated analyses can be expected to give only an indication of the overall picture.

More than 50 cases have been diagnosed as healthy for cobalt, where the concentration has been in excess of 0.10 p.p.m. The large majority of these were from unthrifty animals, most of which had received no cobalt supplements and which were affected by unknown complaints apparently not of parasitic origin. Many were from districts where there is no evidence of cobalt deficiency.

Cattle.

All 5 deficiency diagnoses (range 0.051 to 0.080 p.p.m. in animals 9 months and older) have been sustained by subsequent good results from the use of cobalt. Two of 3 " border-line " cases in yearlings, where the figures ranged from 0.094 to 0.105 p.p.m. have subsequently been confirmed by responses to cobalt. In 3 other cases, this time in unthrifty 3 to 4 months calves, figures of 0.079, 0.100 and 0.103 p.p.m. were obtained. In none of these cases has the suspected marginal deficiency been confirmed.

All other samples from unthrifty animals, 9 months or older, which showed figures above 0.13 p.p.m. have been classified as healthy for cobalt.

(2) *Total cobalt.*

Though the correlation between total cobalt and incidence of bush-sickness is good, there appears to be no advantage in determining total cobalt except possibly to assist in interpretation of results in some cases where the liver is of unusual size.

DIAGNOSTIC CRITERIA

On the basis of the reference data here presented, the following criteria are suggested :

SHEEP.

Three months and older, below 0.06 p.p.m. cobalt deficiency, above 0.10 p.p.m. adequate cobalt.

Less than 3 months, below 0.04 p.p.m. suspected cobalt deficiency, above 0.08 p.p.m. adequate cobalt.

CATTLE.

Nine months and older, below 0.05 p.p.m. cobalt deficiency, above 0.12 p.p.m. adequate cobalt.

Less than 9 months, data inadequate, but lower levels are indicated, especially in new-born calves.

Evidence from the response to cobalt supplements of other animals on farms where figures intermediate between these limits have been obtained in diagnostic work suggests that values below 0.08 p.p.m. in sheep 6 months and older and below 0.10 p.p.m. in cattle 9 months and older are indicative of sub-optimal amounts of cobalt.

COPPER

Figures for copper have been recorded in Tables I to V. The main purpose of the analyses for copper was to ensure firstly that bush-sickness in the North Island of New Zealand was not associated with copper deficiency as in South Australia, and secondly to ensure that there was no possibility of copper deficiency in diagnostic cases referred to this Laboratory. The copper levels in bush-sick animals (Table III) confirm Aston's early findings (36) that typical bush-sickness on non-peaty pumice lands in the North Island is *not* associated with copper deficiency. On the contrary, the average copper level in bush-sick sheep 3 months and older was 460 p.p.m. as compared with 320 p.p.m. for healthy untreated animals from healthy districts, and 406 p.p.m. for healthy cobalt treated from healthy districts. The higher level in bush-sick areas is confirmed from analyses of livers of healthy treated animals from these same areas, where the average copper level found for sheep 3 months and older was 556 p.p.m. The figure of 460 p.p.m. for bush-sick sheep would be increased if the calculations were made on fat-free basis as several of the samples were from animals with enlarged fatty livers. In the South Island, Dixon (37) at Morton Mains and Askew (6) at Glenhope have obtained comparable figures for copper in ailing sheep.

Several cases of enzootic icterus in animals from the Mamaku Farm have been associated with very high copper figures in the liver, in excess of 1,000 p.p.m. on dry basis. Analyses by Piper's modification (38) of Sylvester and Lampitt's method (39) showed copper levels in two pasture samples from Mamaku control paddock No. 9 of 12.4 and 14.0 p.p.m. This indicates that the intake of copper at Mamaku may be higher than in the cobalt deficiency areas of Western Australia (40) where the average copper content in typical cases was only 3.7 p.p.m. (9.9 for holdings unaffected by enzootic marasmus).

Among diagnostic samples handled by the writer only one case of suspected combined cobalt and copper deficiency was noted, from Waipapa in North Auckland. This and any cases of suspected simple copper-deficiency, were referred to Dr. I. J. Cunningham for his copper investigations.

As published work on copper contents of livers indicated divergent opinions as to what constituted normal levels in the livers of sheep and cattle, the samples obtained for the cobalt survey of healthy districts were analysed for copper content for reference purposes.

In sheep there appears to be no very significant change in copper concentration from birth (average 288 p.p.m.) through to maturity (average 323 p.p.m., Table I), though the levels at weaning may be somewhat lower. On the other hand, livers of new-born and bobby calves show levels of copper similar to sheep but the copper concentration drops appreciably from birth through to yearling stage, a conclusion which has been arrived at by many other workers (41) to (46). The total copper content in consequence remains relatively constant. This investigation has revealed some low figures for copper in the livers of apparently healthy cattle beasts from country in which cobalt deficiency does not occur.

IRON

Enzootic marasmus in Western Australia (13), "coast disease" in South Australia (12), and some cases of "salt-sick" in Florida (47), are associated with marked deposition or accumulation of iron in the liver, kidney and spleen. In coast disease the liver shows particularly high deposition of iron, the average reported by Marston *et al.* (12) for 13 untreated weaners showing 16,100 p.p.m. and for one ewe 12,000 p.p.m. iron. Nine sheep affected by enzootic marasmus showed in Underwood's work (13) an average iron content of 2,380 p.p.m. In order to compare New Zealand results with these findings, iron was determined on most of the samples analysed for cobalt and copper.

The average for 29 North Island (N.Z.) bush-sick sheep (Table III) was 360 p.p.m. (range 80 to 853) as compared with 180 p.p.m. (range 89 to 457) for healthy untreated sheep aged 3 months and older from healthy districts. Animals 6 months and older kept healthy on bush-sick country with cobalt supplements showed intermediate values, average 215 p.p.m. (range 110 to 303). Results in Table I show that the concentration and total amount of iron in the liver normally drop from birth through to weaning.

The average iron content in the livers of 20 bobby calves from healthy cows was 1,290 p.p.m. (range 67 to 4,380), compared with 300 p.p.m. (range 137 to 644) for 21 healthy yearlings and mature cattle beasts.

The low figures for iron in the livers of North Island bush-sick sheep (average 360 p.p.m.) are similar to those found by Dixon (37) in Morton Mains disease in the South Island of New Zealand and are in marked contrast with the high figures reported for cobalt deficiency ailments in Australia.

In coast disease the copper content ranged from 5 to 10 p.p.m. (12), in enzootic marasmus 14 to 22 p.p.m. (40), and in bush-sickness 60 to 1,220 p.p.m. (Table III), suggesting an inverse relation between concentration of copper and extent of accumulation of iron in the livers. Underwood and Beck's results (40) indicate that the excessive stores of iron in the tissues of affected animals are used up during recovery from enzootic marasmus while being treated with cobalt only. It would, therefore, appear that the iron mobilizing effects of cobalt and copper are supplementary, with copper playing the dominant role. Thus iron is immobilized in the liver when there is either a severe deficiency of copper, or a moderate deficiency of copper and a deficiency of cobalt.

CONCLUSIONS

(a) Good correlation is evident between both cobalt concentration and total cobalt content in the liver and incidence of bush-sickness in sheep and cattle. In general the correlation is considered to be better than was obtained from analysis of pastures. There appears to be no additional advantage from determining total cobalt as well as concentration.

(b) Sheep affected by facial eczema, copper deficiency, enzootic icterus, unthrifty sheep and cattle from healthy areas, and cattle affected by copper deficiency, show cobalt levels similar to healthy animals, confirming that the correlation is with cobalt deficiency and not with unthriftiness due to any cause.

(c) Four abnormally low figures for cobalt in livers of healthy sheep have been observed but all appear to be from marginal deficiency areas.

(d) The average figure for copper in livers of typical bush-sick sheep is somewhat higher than normal for New Zealand and very much higher than typical examples of enzootic marasmus or coast disease.

In the normal sheep livers examined there appears to be no significant change in copper concentration from birth through to maturity. In calves the copper concentration drops appreciably from birth to yearling stage.

(e) The average iron content in the livers of bush-sick sheep is higher than that found in healthy normals but accumulation of iron is very slight compared with enzootic marasmus and coast disease. Similar trends are found in the limited data for cattle. The iron mobilizing effects of cobalt and copper appear to be supplementary, with copper playing the dominant role.

(f) Neither copper nor iron contents can be used as an indicator of cobalt deficiency in New Zealand.

(g) For diagnostic purposes the following criteria have been successfully employed.

(i) For sheep 3 months and older, concentrations of cobalt below 0.06 p.p.m. indicate cobalt deficiency, above 0.10 p.p.m. sufficiency of cobalt.

(ii) For yearling and mature cattle beasts figures below 0.05 p.p.m. indicate cobalt deficiency and above 0.12 p.p.m. adequate cobalt.

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TABLE I. HEALTHY SHEEP AND CATTLE WHICH HAVE RECEIVED NO COBALT SUPPLEMENTS

	Number of Samples.			Concentration (p.p.m.).						Number of Samples.			Total Metal (mg.).					
				Cobalt.		Copper.		Iron.					Cobalt.		Copper.		Iron.	
	Co	Cu.	Fe.	Range.	Av.	Range.	Av.	Range	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
(A) SHEEP.																		
(I) *Healthy Districts.*																		
1. Foetal lambs from healthy ewes																		
(a) 3 months	4	4	4	.051-.107	.074	67-132	111	107-3920	2330	2	2	2	0006 .0010	.0008	1.0,1.2	1.1	32.26	29
(b) full term.	4			.073-.091	.083													
2. New-born lambs from healthy ewes.	4	4	4	.060-.117	.083*	163-440	288	880-8400	3630	4	4	4	.0008-.0016	0014	2.9-6.0	4.6	20-114	53
3. Fourteen days lambs, as last.	2	2	2	.088,.096	.092	110,150	130	1720,3190	2460	2	2	2	.0029,.0029	.0029	3.7,4.6	4.2	76,98	87
4. Four and eight weeks lambs, as last.	4	4	4	.107-.141	.120	64-285	189	104-1750	554	4	4	4	.0052-.0149	.0095	6.7-24.8	14	8.5-86	32
5. Sheep 3 months and older.	70	70	46	.063-.322	.167	101-1370	323	89-457	182	67	67	43	.010-.073	.026	15-117	47	11-64	26
(II) *Apparently Healthy*†, *untreated, from borderline areas.*																		
1. North Auckland (1 year).	2	2	2	.029,.036	.033	500,150	325	119,252	186									
2. Taranaki (4 months).	2	2	2	.050,.056	.053	288,382	335	108,194	151	1	1	1	-..008		-,52		-,26	
(B) CATTLE.																		
1. 7 months to full term foetus from healthy cows, no cobalt supplements.	7	7	7	.097-.162	.127	406-724	503	2080-6840	3630	7	7	7	.0050-.0122	.0078	24-49	31	121-513	228
2. Healthy new-born calves, as last.	20			.031-.147	075					20			004-.030	.012				
3. "Bobby" calves (2 to 5 days) as above.	18	17	14	.074-.226	.119	139-502	337	67-4360	1270	14	13	11	.007-.035	.016	21-80	46	10-689	181
4. Cattle beasts, 6 months and older.	33	33	21	.122-.398	.240	6‡-191	49	137-644	302	6	6		.129-.168	.146	6-116	63		

* Figure of 0.50 reported in early work (16) suspected too high due to contamination from high cobalt samples analysed about the same time.
† Apparently healthy though from farms with histories of ailment consistent with cobalt deficiency.
‡ Though these animals were described as healthy, Cunningham's investigations (14) raise a query about the copper status of several.

TABLE II.

HEALTHY SHEEP AND CATTLE WHICH HAVE RECEIVED COBALT SUPPLEMENTS

	Number of Samples.			Concentration (p.p.m.).						Number of Samples.			Total Metal (mg.).					
				Cobalt.		Copper.		Iron.					Cobalt.		Copper		Iron.	
	Co	Cu.	Fe.	Range	Av.	Range.	Av.	Range.	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
(A) SHEEP.																		
(I) *Healthy Districts.*																		
(a) *Access to Cobalt Licks* (Small amounts of Cobalt). Sheep 3 months and older.	19	16	8	.114-.348	.216	70-768	282	84-466	182	18	15	7	.018-.071	034	12-145	42	10-85	28
(b) *Cobalt drenched* (Small amounts of Cobalt included in drench). Sheep 3 months and older.	7	6	5	.128-.280	.182	166-1630	736	122-1740	719	5	4	3	.016-.041	.027	24-188	86	18-53	33
(c) *Combining* (a) *and* (b).	26	22	13	.114-.348	.207	70-1630	406	84-1740	388	23	19	10	.016-.071	032	12-188	51	10-85	30
(II) *Cobalt Deficiency Areas.*																		
(a) *Cobalt topdressed pastures.*																		
1. New-born lambs from healthy ewes	8	8	8	.105-.200	.151	40-320	167	250-1070	518	8	8	8	.0018-.0052	.0033	0 8-6.5	3.6	4-35	13
2. Healthy 3 weeks lamb as (1).	1	1	1	.112		149		83		1	1	1	.0038		5.0		3	
3. 4 years old ewes.*	9	8	8	.078-1.17	.485	169-706	476	110-303	225	9	8	8	.019-.204	.095	43-153	98	20-77	49
(b) *Access to licks containing small amounts of Cobalt* (wethers)	2	2	2	.168,.220	.194	432,720	576	348,130	239	2	2	2	.025,.022	023	65,71	68	52,13	32
(c) *Cobalt drenched.*																		
1. New-born lambs from healthy ewes drenched. 1 mg. Co. twice weekly. (Pdk. 9).	2	2	2	.085,.126	.106	212,280	246	235,1420	828	2	2	2	0011,.0031	.0021	2.8,6.9	4.8	3.1,35	19
2. Healthy 18 days lamb as above.	1	1	1	.116		143		238		1	1	1	.0048		6.0		9.9	
3. 6 months lambs drenched several weeks as in (1).	4	4	1	.097-.305	.162	445-738	603	182		4	4	1	.012-.035	.019	52-85	70	21	
4. 4 years ewe, as last, drenched 10 months	1	1	1	.408		896		262		1	1	1	106		233		68	
5. 18 months wethers, 140 mg Co once monthly, 9 months.	2	2	2	.417,.497	.457	473,830	652	203,152	178	2	2	2	.134,.117	.125	151,196	174	65,36	51
6. Combining (a) 3, (b) and (c) 3-5	18	17	14	.078-1.17	.357	169-896	556	110-303	215	18	17	14	.012-.204	.074	43-233	105	13-77	46
(B) CATTLE.																		
Healthy Districts.																		
Access to Cobalt Licks.																		
1. "Bobby" calves from cows with access to licks.	7	7	6	.075-.141	.107	159-532	352	263-4380	1400	4	4	3	.008-.016	.013	32-60	46	30-70	45
2. 4 years ox.	1	1	1	.184		79		149										

* Most of these ewes died from lambing trouble but none showed evidence of cobalt deficiency

TABLE III

BUSH-SICK SHEEP AND CATTLE (UNTREATED)

	Number of Samples			Concentration (p.p.m.)						Number of Samples			Total Metal (mg.).					
				Cobalt		Copper.		Iron					Cobalt		Copper.		Iron.	
(1) SHEEP.	Co	Cu.	Fe.	Range	Av	Range	Av.	Range	Av	Co	Cu	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
(a) Foetal lamb from bush-sick ewe.	1			.021						1			.00007					
(b) New-born lamb from bush-sick ewe.	1	1	1	.034		450		2720		1	1	1	.00029		3.8		23	
(c) Sheep 5 months and older	30	29	29	.008-.054	032	60-1220	460	80-853	363	26	25	25	.0012-.0122	.0046	5-159	66	18-159	56
(2) CATTLE																		
Bush-sick yearlings.	3	3	3	.025, .038 .038	034	122, 92, 113	109	746, 420, 987	718	1	1	1	.037 (.038 p.p.m.)		90 (92 p.p.m.)		41 (420 p.p.m.)	

Table IV. Sheep and Cattle Affected by Ailments Other Than Bush-sickness

	Concentration (p.p.m.).										Total Metal (mg.).								
	Number of Samples.			Cobalt.		Copper.		Iron.			Number of Samples.			Cobalt.		Copper.		Iron.	
Sheep.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.		Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
(1) ***Facial Eczema.***																			
Sheep 3 months and older.	6	6	6	.073-.132	.091*	272-685	532	92-271	176		3	3	3	.010-.016	.013	35-121	71	15-43	26
(2) ***Copper Deficiency.***																			
(a) New-born lambs from ewes in copper deficient areas.	5			.047-.132	.099						5			.0011-.0016	.0014				
(b) Ewes.	4			.166-.282	.205						4			.025-.059	.042				
(3) ***Enzootic Icterus.***																			
(a) Country normally healthy for cobalt (6 months and older).	10	14	13	.057-.243	.122*	1080-3520	2160	90-1610	1020			4	4			113-207	144	80-144	107
(b) Bush-sick country.																			
1. Apparently cobalt deficient also.	1	1	1	.018*		1650		536											
2. Cobalt supplements.	4	4	4	.051-.098	.077*	1450-2140	1820	620-1390	1020		1	1	1	.0075		165		54	
(4) ***Fed Excess Copper.***†																			
1g. $CuSO_4.5H_2O$ daily till death.	5	6	6	.040-.110	.068*	2090-4320	3030	55-1370	468										
Cattle.																			
Copper Deficiency.																			
20 months and older (no Cobalt supplements).	8			.134-.307	.228						8			.115-.573	.379				

* Analyses by earlier less reliable method (3), liable to be low.

† Cases of experimentally induced jaundice—M. Buddle's experiments at Wallaceville (48).

TABLE V.

OTHER ANIMALS

	Number of Samples			Concentration (p.p.m.).						Number of Samples.			Total Metal (mg.).					
				Cobalt.		Copper		Iron.					Cobalt.		Copper.		Iron.	
	Co.	Cu.	Fe.	Range	Av	Range	Av	Range.	Av.	Co.	Cu.	Fe.	Range.	Av.	Range.	Av.	Range.	Av.
A. *Goats.*																		
(1) Healthy wild kid.	1	1	1	.222		110		160		1			.0057		2.8		4.1	
(2) Suspected cobalt deficiency.																		
(a) Kid goat.	1	1	1	.203		17		112					.014		1.2		8	
(b) Nanny goat (4 years).	1	1	1	.170		18		184					.016		1.7		17	
B. *Pigs.*																		
Healthy 4-9 months.	8	8	8	.068-.190	.102	13-37	23	428-804	641	8	8	8	.007-.040	.014	1.2-5.2	3.0	40-196	88
C. *Horse.*																		
Healthy.	1			.196														
D. *Rabbit.*	1			.34		16		720					.0032		0.15		6.7	
E. *Rats.*																		
Healthy untreated.	18	12	22	.14-.24	.18*	12-18	15	240-1280	725				.00016-.00047	.0003	.012-.055	.039	0.30-1.9	1.2

* See (49). Figure of 1.4 p.p m. reported in earlier work for Josland (15) suspected of contamination from high cobalt samples.

TABLE VI. CORRELATION OF COBALT CONTENT OF MAMAKU AND WAIMIHA PASTURES AND COBALT CONTENT OF LIVERS WITH BUSH-SICKNESS

Description	Number of Samples	Cobalt content (p p m) on dry matter			
		Pasture		Liver	
		Range	Yearly Av	Range	Average
Mamaku.					
Bush-sick* lamb	4	0.04 0.07	0.06	0.039-0.045	0.042
,, hoggets	12	0.04-0.07	0.06	0.008-0.040	0.024
,, ewes	3	0.04 0.07	0.06	0.034-0.054	0.044
Healthy 2-tooth	1	0.06-0.11	0.08		0.103
Healthy 4 yrs ewes†	1	0.05-0.62	0.16 (approx)		0.38
,, ,,	3	0.05-1.2	0.18 ,,	0.078 1.17	0.61
,, ,,	2	0.08 0.62	0.27 ,,	0 25-0.29	0.27
,, ,,	1	0.08 2.8	0.63 ,,		1.05
Waimiha					
Bush-sick ewes	6	0.04-0.08	0 06 ,,	0.033-0.045	0.040
Healthy ewes	6	0.10 1.03	0.50 ,,	0.35-0.80	0.52

* About half of these bush sick animals died, the balance being killed in a weak or unthrifty condition and showing typical symptoms of severe deficiency

† These ewes died from lambing trouble but were otherwise in good condition Their weight and general health records ruled out the possibility of bush-sickness

BACILLUS MESENTERICUS: AN ASSAY ORGANISM FOR PENICILLIN

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(*Received for publication, 13th August, 1947*)

Summary

The use is described of *Bacillus mesentericus* as a test organism in the plate method of penicillin assay. Its advantage over *Staphylococcus* are indicated.

INTRODUCTION

IN the determination of the potency of penicillin solutions by the Heatley plate method it is usual to employ a suitably sensitive strain of *Staphylococcus*. This organism is, however, subject to certain disadvantages; of these the more important have been noted by Foster and Woodruff (1943 a), (1944). Most of the drawbacks mentioned by these authors have been experienced in this laboratory and more especially has it been found that staphylococci show a physiological variability from time to time. In addition the growth is usually not sufficiently opaque to give a good zone contrast and many strains tend to flocculate markedly in broth thus finally producing irregular growth in test plates. This has been observed more particularly with the culture of *S. aureus* strain " H " in our possession.

Foster and Woodruff (1943 b) in introducing the use of *Bacillus subtilis* as a test organism claim that it obviates most of the failings of staphylococcus.

Two years ago when it became evident at Wallaceville that *S. aureus* was not entirely suitable for penicillin assays it was decided to test a number of organisms of the *B. subtilis* group. Of these our strains of *B. subtilis, B. mycoides* and *B. megatherium* proved insufficiently sensitive and failed in other respects to fulfil the requirements of the test. A fourth type, namely, *B. mesentericus,* gave such successful results that it has since been employed here in routine penicillin determinations.

THE STRAIN

In 1942, during the course of a bacteriological examination of some pig feed, *Bacillus mesentericus* was isolated together with numbers of other typical soil flora. This was the source of the strain now used at Wallaceville for assay work. Culturally and biochemically it duplicates the characters assigned to *Bacillus mesentericus vulgatus* by Topley and Wilson (1936) except that nitrates in broth are not reduced. Bergey (1939) states, however, that nitrate reduction may or may not occur within this species.

THE ASSAY METHOD

The assay procedure adopted here differs in no essential respect from those usually employed. Ordinary 2.5 per cent. nutrient agar is used as a medium; this is distributed in 100 ml. lots in flasks. An 18 hour nutrient broth culture of *B. mesentericus* is used as an inoculum at the rate of 0.5 ml. of culture per 100 ml. of agar, and is added to the pre-melted agar at 46° C. This gives a density of organisms such that after incubation the colonies are almost but not quite confluent. A quantity of 12 ml. of the sown agar is apportioned to each plate, after which, without preliminary drying, the test solutions are added. The plates are incubated at 37° C. for 14 to 18 hours.

Incubation produces a highly opaque bacterial growth; the zone margins are clearly defined and the marked contrast between areas of growth and of non-growth facilitates accurate measurement of the zone diameters. The sensitivity of *B. mesentericus* between 0.5 and 1.5 units per ml. is roughly equivalent to that of *S. aureus* strain " H," but while the sensitivity curve follows the usual shape it tends to be flatter than that of the latter test organism. Penicillin potencies as low as ⅛ unit per ml. are easily determined using the cork-borer (No. 3) method of agar-excision; this level of concentration gives a zone diameter of about 12.2 mm., while the diameter at a concentration of 5 units per ml., which is about the upper practicable limit, is approximately 26 mm.

The advantages of *B. mesentericus* may be summarized as follows: (1) physiological and cultural stability in time and in different batches of media; (2) excellent definition of inhibitory zones; (3) absence of the ring formation which frequently results from bacterial lysis in the case of *S. aureus;* (4) homogenous growth in nutrient broth without flocculation or pellicle formation (no surface growth appears until 4 to 6 days); (5) it is non-pathogenic; (6) owing to rapid growth, zones are measurable after from 8 to 10 hours of incubation.

On blood agar *B. mesentericus* is strongly Beta-hæmolytic and this property suggests it would be a suitable organism for use in the tube assay method of Rammelkamp (1942).

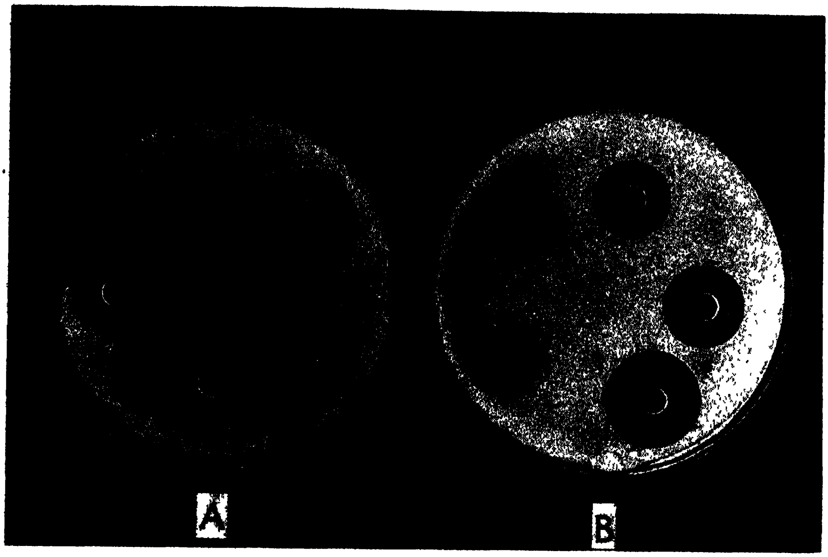

FIG. 1

The illustration (Fig. 1) shows an assay using (A) *S. aureus* strain "H" and (B) *Bacillus mesentericus* in which the zone clarity can be seen down to the lowest concentration of penicillin. It should be stated that the particular plate of *S. aureus* was selected as being the best of several dozens whereas that of *B. mesentericus* is quite typical.

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CONTROL OF HALO-BLIGHT OF BEANS

By W. D. REID, Plant Bacteriologist, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 30th July, 1947*)

Summary

In 1945-46 and 1946-47 seasons dwarf beans infected with halo-blight (*Pseudomonas medicaginis* var. *phaseolicola* Stapp and Kotte) were treated with Bordeaux (6-8-100) and Cuprox (copper oxychloride, 5-100).

Treatments consisted of two, three and four applications of each spray material.

Because of low rainfall, incidence of halo-blight in 1945-46 season was low but all treatments reduced infection to a very small amount. In 1946-47 season results showed that three and four applications reduced disease to a negligible amount but that two applications were not as effective.

Bordeaux and Cuprox were of approximately equal value for control of halo-blight.

Green-pod plus seed yields of treated plots were approximately double those of untreated plots.

INTRODUCTION

IN a previous paper (Reid and Taylor, 1945) results of copper spray treatments for control of halo-blight (*Pseudomonas medicaginis* var. *phaseolicola* Stapp and Kotte*) were given. These showed that four applications each of Bordeaux and of Cuprox (copper oxychloride 50 per cent. copper) reduced infection to a negligible amount and that two and three applications, though not as effective, appreciably limited infection. To check these findings, results of which are reported in present paper, further trials were carried out in 1945-46 and 1946-47 seasons.

METHODS

In each season an area of ground 140 feet by 60 feet was sown with Masterpiece dwarf bean seed in 29 drills 24 inches apart. After germination plants were thinned to 3 in. and the area was divided into 49 plots each of four rows 16 feet in length. Treatments were arranged in the form of a 7 x 7 latin square. Every fourth row served temporarily as an infection row, and was removed as soon as halo-blight was established. When plants had formed the first true leaves, the three permanent rows of each plot, with the exception of check plots, received a first application of spray material. After the spray had dried, the whole area, including infection rows and check plots, was sprayed with a water suspension of halo-blight bacteria. In the 1945-46 season infection rows were removed on 14th January and in 1946-47 season on 30th December.

Treatments consisted of two, three and four applications of (1) Cuprox (5 lb. per 100 gallons water) and (2) Bordeaux (6 lb. copper sulphate, 8 lb. hydrated lime, 100 gallons water) applied with a knapsack pump.

Times of application were arranged to give maximum protection from seedling stage to time of picking green pods, a total period estimated as approximately 36 days. This period was divided according to the number of applications intended; thus there were intervals of 18, 12 and 9 days between applications for the two-, three- and four-spray treatments respectively. In the 1945-46 season the first application of all treatments was given on 4th January and the last, that of the four-spray treatment, on 4th February. In the 1946-47 season the first application was given on 11th December and the last on 8th January.

The amount of spray required for a good cover increased with age of plants, the first application taking approximately ¾ gallon for seven plots of a treatment and the last 3 gallons for the same area of 74.6 square yards.

Periodical counts of plant and pod infection were recorded. Infected plants were graded 0 to 5 (Reid, 1945) relative to amount of disease present, 0 representing freedom from disease and 5 severe infection and possibly stunting of plant. Ten plants distributed through each row were so graded and the "infection index" in Table I gives average plant infection in each treatment. Pods of marketable size were picked on two occasions and were weighed, counted and recorded as infected or free from disease. In the second season further growth of pods occurred after the second picking and these were later harvested and the seed weighed.

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* In previous paper designated *Pseudomonas medicaginis* (Sackett) Dowson. According to the most recent classification, this species is now *P. phaseolicola* (Burkholder) Dowson.

RESULTS

1945-46 Season

Early examination of plants in infected rows showed an even distribution of halo-blight over the area. In this season there was no further increase in incidence after the initial infection. On 23rd January, 19 days after inoculation, check plots showed an average "infection index" of 3.2 and treated plots varied from 0.4 to 0.5 for Bordeaux and 0.6 to 0.9 for Cuprox treatments. The greater the number of spray applications the smaller was the index figure. The amount of disease on harvested pods indicated a similar gradation; checks showed 14.2 per cent. infected on 7th February and 8.9 per cent. at picking on 18th February; treated plots averaged 0.2 per cent. and 0.05 per cent. infection at these two picking dates respectively. Differences between results of both plant and pod infection of check and treatment plots were highly significant but no significant differences were apparent between treatments. Green-pod yields varied from 186 ounces per plot for checks and three- and four-spray Cuprox treatments to 216 ounces for the two-spray Bordeaux treatment. This difference in yield of 30 ounces was just significant at the 5 per cent. level.

1946-47 Season

Results of the 1946-47 season are given in Table I. In the three- or four-spray plots, infection regularly consisted of a single small lesion on each pod, whereas in check plots many lesions or extensive infected areas occurred on majority of pods. Pods from check plots were useless for marketing.

TABLE I. EFFECTS OF 2, 3 AND 4 APPLICATIONS OF COPPER SPRAY MATFRIALS ON CONTROL OF HALO-BLIGHT OF BEANS

Treatment.	Number of Applications.	Halo-blight Infection, Treatment Averages.			Yields; Plot Averages	
		Plant Infection Index 13/1/47.	Pods; Per cent Infection 21/1/47.	27/1/47.	Pods; Oz per Plot 21 & 27/1/47.	Seed; Oz. per Plot 26/2/47.
Cuprox 5-100	2	0.3	1.48	21.58	408.6	37.3
	3	0.2	0.23	10.91	390.1	33.1
	4	0.1	0.22	6.43	365.9	34.7
Bordeaux 6-8-100	2	0.9	2.23	27.94	412.4	39.8
	3	0.1	0.45	10.59	376.0	38.4
	4	0.1	0.26	8.77	394.8	37.8
Check. No Spray	–	4.1	75.58	56.88	204.9	4.1
Standard Error		0.1	0.93	2.30	25.1	4.3
Diff. for Significance at 5 per cent. Level		0.3	2.67	6.65	72.5	12.5

DISCUSSION

Although the amount ot disease present in the 1945-46 trials was small, as shown by the check plots, treatments markedly reduced plant and pod infection. The low incidence of halo-blight in a susceptible variety is unusual and in 1945-46 was apparently associated with low rainfall. In the three months covered by the 1945-46 trials the rainfall was 2.18, 0.82, and 0.51 inches, while in the following season for the three months of the trial it was 5.33, 2.91 and 2.67 inches. The average pod infection in check plots in the two periods were 11.5 per cent. and 66.2 per cent. respectively.

The results for 1946-47 also show that spray applications effectively checked bacterial infection of plants and pods. At the same time heavy infection in check plots decreased pod yield to approximately half that of treated plots. Seed yield was similarly affected though in this case the difference was greater. Bordeaux and Cuprox were of nearly equal value in preventing infection of plants and pods. The number of applications, however, brought about marked differences in amounts of infection. The two-spray figures of both Bordeaux and Cuprox on 27th January and of Bordeaux on 13th January, were significantly greater than those of other treatments on these dates. There were no significant differences between results of three- and four-spray treatments. The two-spray treatments show higher pod yields than do the three- or four-spray treatments. The differences might have arisen from variation in plant damage associated with number of spray applications. However, they are not significant at the 5 per cent. level, and from a practical point of view, the advantages of the higher yields are offset by the greater amount of disease present.

Whilst the above results are highly satisfactory it is probable that under commercial conditions, which would not include infection rows or untreated check plots, spray treatment would give an even better control.

In Table I, records of pod infection on 27th January show much higher figures than those of 21st January. This was because the period between the last application of treatments and picking of pods was unexpectedly prolonged by slow maturing of crop. Thus adequate protection was not maintained during later stages of pod development. This could be rectified either by an additional late spray or by adjusting times of application to give more uniform protection over the whole growing period.

CONCLUSIONS

The results, together with those previously reported (Reid and Taylor, 1945) show that halo-blight of beans can be readily controlled by use of Bordeaux and Cuprox sprays. At least three applications are advisable and the intervals between them should be adjusted to give protection from the seedling stage to crop harvest.

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PRELIMINARY AERIAL DISTRIBUTION TRIALS WITH SUPERPHOSPHATE AND SEED MIXTURES

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INTRODUCTION

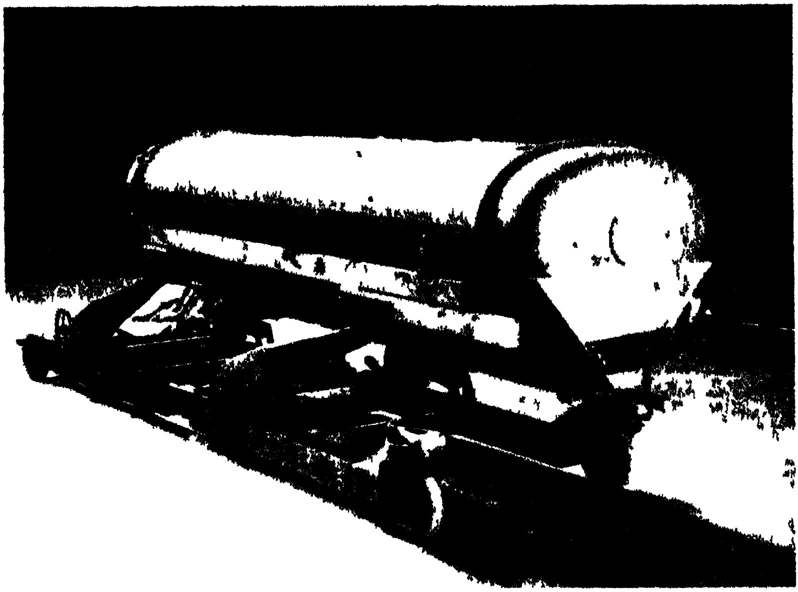
THE problems of combating depletion of pastures and controlling soil erosion and excessive run-off on sown and native hill country grasslands are becoming increasingly acute owing to the cost and scarcity of labour and lack of access. The most immediate conservation problem is improvement of the sward and the most effective large scale remedies are topdressing to improve fertility and seed sowing to provide responsive clovers and grasses. The possibility of using aircraft for these purposes consequently merits full investigation. To be effective the measures of sowing are necessarily coupled with prudent grazing, pest and fire control; trials were therefore made where the latter safeguards were assured. The preliminary aerial fertilizer trials were confined to typical North Island hill country pastures and the seeding trials were carried out on typical depleted South Island high country native pastures.

HISTORY

The possibility of applying fertilizers and seed from aeroplanes led the writer to investigate and formulate proposals which were adopted by the Soil Conservation and Rivers Control Council in 1947. These proposals were further considered at the Council's request by an expert Interdepartmental Committee consisting of the Departments of Works, Air, Scientific and Industrial Research, Agriculture and the Soil Conservation Council. This committee advised that the seed sowing trials be done by Aerodromes Services, Ministry of Works, and the fertilizer trials by the Royal New Zealand Air Force.

FERTILIZER TRIALS

From the beginning it was recognized that the object of these trials was to provide information on the mechanics of distribution, the design and type of aircraft most suitable, and the practicability of the project. Early consideration was given to using concentrated phosphate, but as this was expensive and difficult to procure, it was decided to use



(*R.N.Z.A.F. Photo.*)

FIG. 1.—Fertilizer Hopper adapted from a Reserve Petrol Tank.

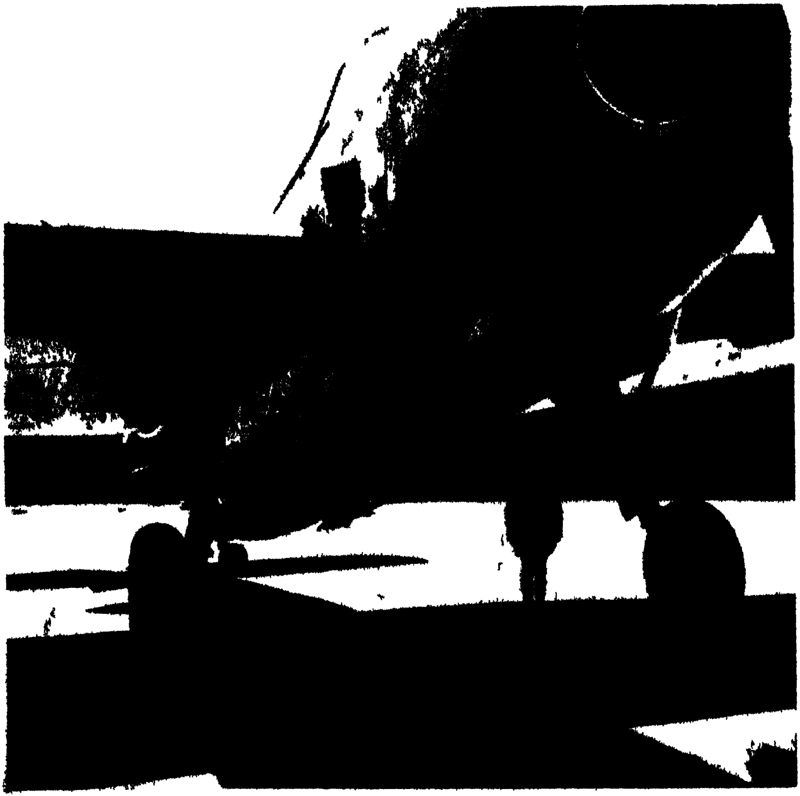


FIG. 2.—Fertilizer Distributor fitted to Avenger Aircraft.

commercial super for the preliminary trials. In the event of the trials being successful concentration of phosphate could then be investigated along with other problems that arose.

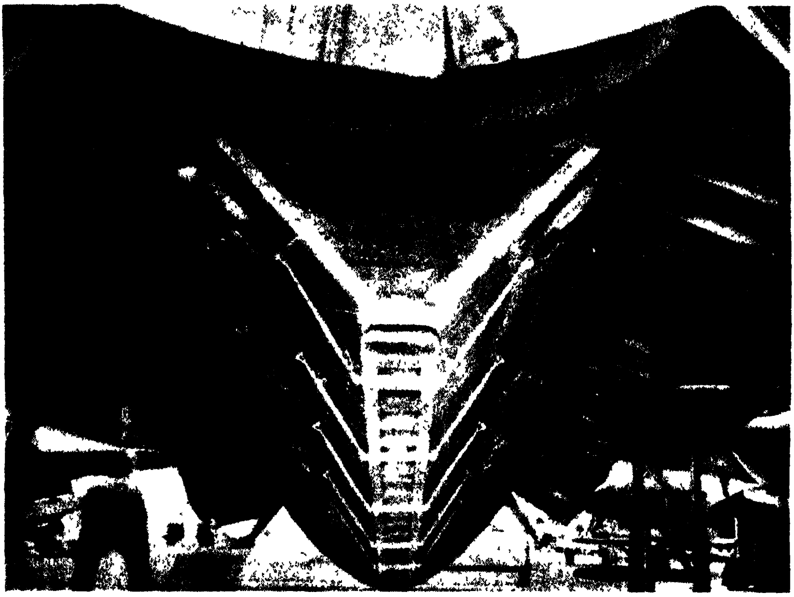
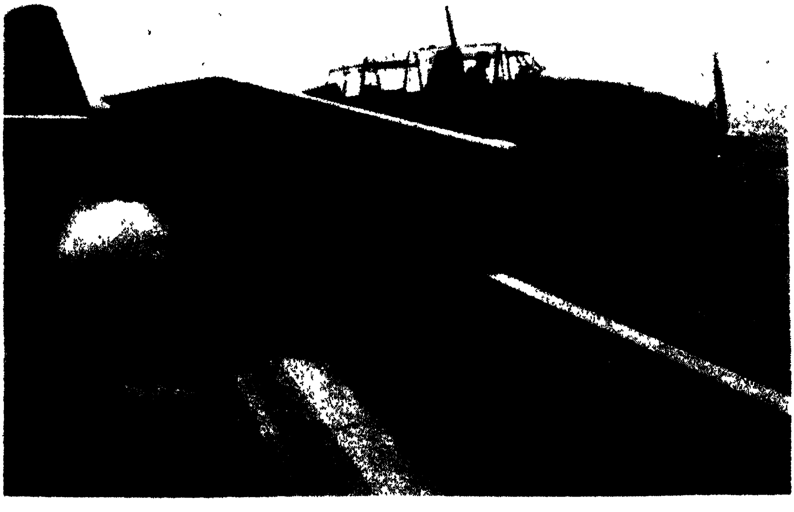


FIG. 3 Close Up view of Fertilizer Hopper (*R.N.Z.A.F. Photo.*)



(*R.N.Z A.F. Photo.*)

FIG. 4.—Hillside Superphosphate being released from an Avenger Aircraft.

The Air Force undertook the preparation of an aircraft for these trials. As ground freight costs were lower than air freight, a large machine designed to carry big loads for long distances was not so desirable for preliminary trials as one carrying smaller loads and capable of operating from smaller aerodromes from which it would virtually lift and distribute the load rather than transport it. As costs of adapting a large aircraft, and investigations into overcoming the dust problem were scarcely warranted in the initial trials, the most suitable machine carrying a relatively small external load was sought ; the most suitable available being an Avenger Torpedo Bomber capable of carrying a 2-ton load. To this machine the Air Force attached a fertilizer hopper (a reserve petrol tank) (Figs. 1, 2 and 3) in the bomb-bay and fitted it with distribution slots and controls designed to discharge at the rate of 2-cwt. per acre a load of one ton of " hill-side " superphosphate, a special granular form of material in which the granules pass a ⅜ in. mesh but are retained on a ¼ in. mesh. With the machine travelling at 125 miles per hour the load had to be dispatched in 37.5 sec.

Calibration Trials with Aeroplane

These trials were carried out at Ohakea by flying across the concrete runways at varying heights with two types of superphosphate—normal commercial and " hillside " superphosphate.

In the first trial carried out in a light wind the cloud of dust from the normal super drifted off the areodrome, and only a small proportion of heavier granules settled on the trial area. Further trials were carried out with " hillside " super, both across wind, into the wind, and with the wind, at elevations of 70 ft., 100 ft., 200 ft., 400 ft. and 600 ft., at a speed of 125 mph. (Fig. 4).

The swathe of " super " pellets was reminiscent of hail both during their fall and on the runway. The width of the swathe varied directly with the height of the aircraft, from 12 yd. width at 70 ft. to approximately 180 yd. at 600 ft. As was expected, cross winds carried the super very appreciably leewards. At 400 ft. elevation a very light breeze deflected the falling super by half the width of the runway (40 yd.).

During the trials sampling boxes similar to those to be used in the later trials at Raglan were difficult to locate so as to completely sample the swathe of super to be dropped in any one run.

However, the most effective sampling was done by sweeping up the super from the 10 sq. yd. hexagons of concrete with which the runway is paved. It was thus possible to measure accurately the transverse and longitudinal distribution of the fertilizer.

The following weights from alternate hexagons along the centre of the line of flight provided a check on the continuity of flow :—

From 400 ft. flying height : 2.45, 2.49, 2.60, 2.57, 2.16, 2.55, 2.37, and 2.36 cwt. per acre.

From 600 ft. flying height : 2.35, 2.12, 2.13, 1.83, 1.90, 1.70 cwt. per acre.

The transverse distribution was carefully checked at 17 ft. 4 in. intervals across the line of flight and recorded as follows :

From 400 ft. flying height : average of pairs of samples. .01, .16, .47, 1.04, 1.78, 2.58, 2.54, 1.82, 1.25, .65, .31, .02 cwt. per acre.

From 600 ft. flying height : .03, .07, .10, .17, .28, .39, .61, 1.08, 1.43, 1.83, 2.13, 1.38, .73, .51, .42, .25, .14, .08, .06, .01 cwt. per acre. (Fig. 5).

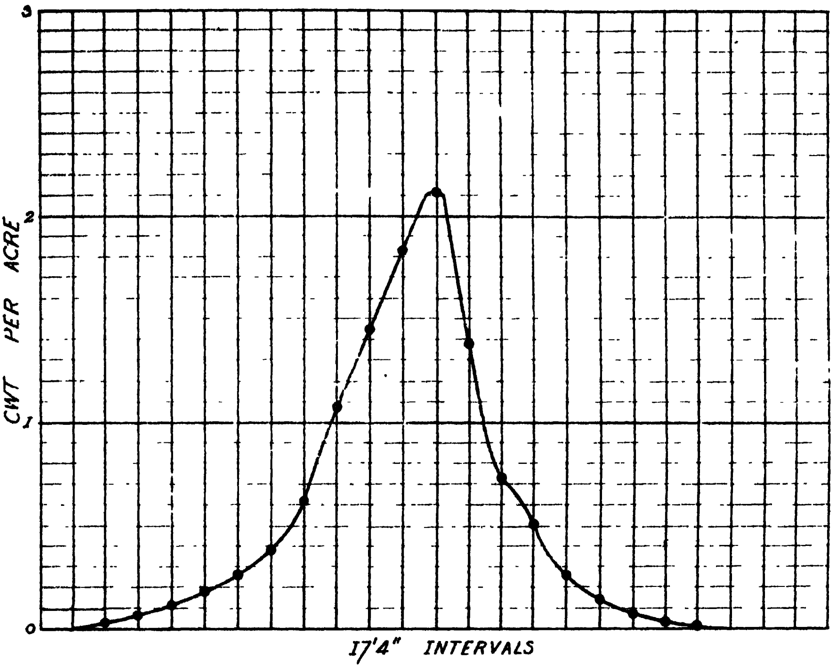


FIG. 5—Distribution of "Hillside" Superphosphate from 600 ft. flying height

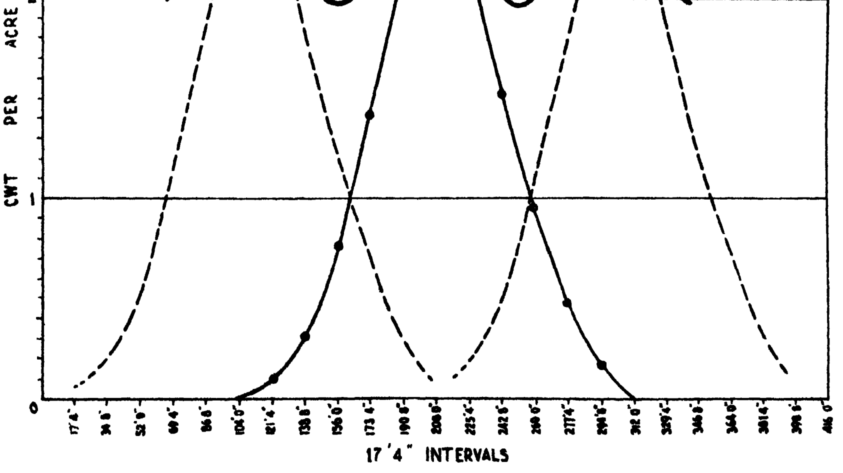


FIG. 6.—Distribution of "Hillside" Superphosphate from 400 ft. flying height, and Effective Distribution in parallel flights 90 ft. apart.

From a graph of these distributions it was calculated that at 400 ft. elevation a spread of 2 cwt. per acre and over was obtained over a swathe of 50 ft., and that by making successive runs at 90 ft. intervals, a top-dressing of from a minimum of 2 cwt. per acre to a maximum at the centre of each flight of 2.5 cwt. per acre could be obtained, providing that the wind did not vary. (Fig. 6). Errors caused by variable winds, had, however, to be accepted in practice on hill country.

In the practical trial, which followed, the required 2-cwt. per acre distribution was closely approximated over a width of 50 ft. at 400 ft. elevation, a suitable flying height for hill country. From a study of the graph it was determined that the aircraft would be required to fly parallel courses 90 ft. apart to ensure that the minimum dressing of 2-cwt. per acre was applied on the periphery with a maximum of 2½ cwt. per acre in the centre of the strip.

The most remarkable outcome of the trials was the distribution effect of the slipstream on the falling particles. This, superimposed on the normal dispersive eddies created by the falling particles, gave unexpectedly effective and wide distribution of the granulated "hillside" superphosphate.

The preliminary trials proved that a sufficiently satisfactory ground distribution of granulated "hillside" super was possible from a fertilizer distributor fitted to an Avenger aircraft flying at a height and speed suitable for hill country. Rapidly changing strength and direction of wind would cause vagaries in the distribution of fertilizer to be overcome only by choosing suitable weather for field scale trials. The expeditious loading and delivery of the fertilizer coupled with the satisfactory spread warranted further trial on a field scale in order that costs and practicability could be further investigated.

TABLE I. SIEVE ANALYSES—BULK SAMPLES

Superphosphate used in Ohakea trials, 21.9.48

Sieve Number.	Sieve Hole Size. (in.)	Percentage of Fertilizer Retained on Each Sieve.	
		Hillside Super.	Commercial Super.
4	0.197	88.9	0.0
10	0.0787	7.5	0.0
20	0.0331	0.7	12.6
40	0.0165	0.6	47.5
60	0.0098	0.3	16.0
80	0.0070	0.2	8.5
100	0.0059	0.4	4.25
100	0.0059	0.9	10.3

Raglan Field Trials

Typical hill country with poor access and sloping steeply from 600 ft. to 200 ft. elevation was chosen on the late J. B. Vowles' property at Te Mata, south of Raglan. On this farm a moderately good mixed pasture of ryegrass, cocksfoot, dogstail, danthonia, white clover and subterranean clover had been established during the previous 25 years on hitherto heavy mixed podocarp forest land.

The Air Force photographically surveyed the location and prepared a flying plan and organized 3-way radio communication between ground, air and base staff. Smoke indicators were lit and moved over appropriate distances prior to each flight.

The Avenger aeroplane operated from Rukuhia Aerodrome some 20 miles distant, and after an establishing run was made, came in over the ridge and descended parallel to the slope of the trial area.

Officers of the Soil Fertility Research Station, Department of Agriculture, who undertook to measure the ground distribution and collate the information on the subsequent response to " super ", placed boxes padded with scoured and weighed wool at intervals over the 27 acre trial area. During the operation the ground distribution was directly checked after each run by collecting and counting the granules that fell on a unit square yard, and the information was communicated to the Control Officer.

From the flying point of view the trial was very satisfactory, but unfortunately the superphosphate did not flow freely owing to the high proportion of fine material in the sample. This was apparent in the field, as the first " hail " of pellets which hit the ground in approximately 12 sec. was followed some seconds later by much finer particles and finally dust. However, for the third and final trip the more granular sacks of " super " were chosen and the load dispatched in runs over the target area as originally planned.

The Department of Agriculture reported that :--

1. the wool-padded boxes placed on a series of parallel lines along the proposed lines of flight trapped the falling fertilizer at the average rate of 13.5 g. per sq. yd. or 144 lb. per acre ;
2. from the square yard wire frames used additionally in transverse sampling of the entire area an average of 6.7 g. per sq. yd. or 72 lb. per acre for 49 samplings was obtained.

   Allowing for a 50 per cent. recovery by this method owing to the fine material present, these sample weights compare favourably with the box samplings.
3. the total of 2⅓ tons dropped on the target area of 27 acres indicated an average distribution of 1⅔ cwt. per acre.
4. an analysis of a composite sample of the super from 32 sacks was as follows :--

TABLE II. ANALYSIS OF HILLSIDE SUPERPHOSPHATE USED IN RAGLAN TRIALS

		Retained on	¼ in. sieve	25.1 per cent.
Passed	¼ in. and	,, ,,	1/9 ,, ,,	8.9 ,, ,,
,,	1/9 ,, ,,	,, ,,	1/16 ,, ,,	5.7 ,, ,,
,,	1/16 ,, ,,	,, ,,	1/25 ,, ,,	7.8 ,, ,,
		Passed	1/25 ,, ,,	52.5 ,, ,,

(Only 25 per cent. of this sample was retained on the ¼ in. sieve)

The ground measurements made, correlated well with the total weight dropped when allowance was made for the unexpectedly large proportion of fine material in the sample, some of which probably drifted with the wind off the area.

In view of the fact that by double screening the manufacturers can assure supply of a satisfactory uniform sample of " hillside super " in future, the field trial must be regarded as being highly satisfactory in proving the practicability of aerial topdressing of average New Zealand hill country.

AEROPLANE DISTRIBUTION OF SEED MIXTURES

Further investigation of seed sowing from the air was necessary to determine the practicability of the operation in view of the widespread need to promote soil conservation by introducing legumes onto North Island hill country pastures and grasses and legumes onto South Island high country native pastures. In the past surface sowing trials have proved to be effective overseas (1) (2) and in New Zealand in assisting the recuperation of this country notably at Molesworth, Mt. Pisa and on " The Bluff's " Run.

From earlier experience in the sowing of lupin seed on sand dunes and the application of cobalt and copper sulphate on trace-element deficient areas, A. M. Prichard, pilot to Aerodrome Services, Ministry of Works, designed and fitted a suitable hopper in a Whitney Straight light aeroplane capable of carrying 400 lb. of seed.

A venturi was arranged to effect a forced dispersion of the seed as it flowed from the hopper by gravity, and the controls were designed to regulate the rate of flow according to speed and height of the aircraft and provide a constant 10 lb. per acre distribution of the mixture sown.

Calibration and Distribution Trials

These were carried out at Rongotai Aerodrome using a mixture of grasses and clovers suitable for high country containing clovers (white, red and subterranean), grasses (cocksfoot and brown top) and yarrow.

In the first trial, flying at an altitude of 100 ft. into a 17 miles per hour wind at a speed of 100 miles per hour, the brown top and yarrow components of the mixture drifted down wind by more than 300 yd. and could not be recorded .

Counts were made of all seeds falling in alternate square yards across the line of flight and recorded.

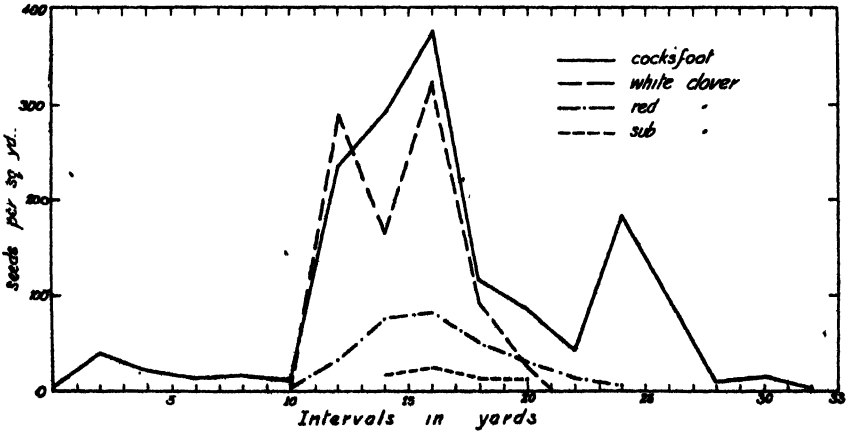


FIG. 7.—Distribution of Seeds released from a plane flying at 100 ft. height into 17 miles per hour wind.

Two trials were subsequently made under calm weather conditions, at 200 ft. and 400 ft. flying height with the composite mixture of grass and clover used in the first trial in order to determine the pattern of distribution under ideal conditions. Counts of seeds were made directly on the Tarmac using a unit square yard in the first trial, and in the second the seed falling was collected on sheets of cloth 20 sq. ft. in area placed at 5 ft. centers across the line of flight.

The ground distribution of the different types of seeds dropped in the three trials are indicated in the following table and in the accompanying graphs (Figs. 7-9).

TABLE III. GROUND DISTRIBUTION OF SEEDS DROPPED FROM AIRCRAFT AT HEIGHTS OF 100 FT., 200 FT AND 400 FT

Seed	Rate per Acre. (lb.)	No. of seeds required per sq. yd. at rate intended.	Width of Distribution (yd.)	Average No of seeds per sq. yd	Average No of seeds per sq yd
Flying height 100 ft					*over 10 yd swathe.*
Cocksfoot	5	500	40	80	221
Red Clover	2	88	20	30	55
White Clover	1	100	14	132	184
Subterranean Clover	1	20	8	18	17
Flying height 200 ft					*over 17 yd swathe.*
Cocksfoot	5	500	48	306	540
Brown Top	½	1550	20	81	84
Yarrow	½	360	22	99	108
Red Clover	2	88	30	41	72
White Clover	1	100	18	50	156
Subterranean Clover	1	15	28	18	18
Flying height 400 ft					*over 39 yd swathe*
Cocksfoot	5	500	76	204	415
Brown Top	½	1550	63	402	788
Yarrow	½	360	60	37	85
Red Clover	2	88	50	88	160
White Clover	1	100	42	171	246
Subterranean Clover	1	15	50	15	26

From the graphs of distribution of individual components of the mixture a compromise was made between effective ground distribution and suitable flying height to determine the distance between the parallel flights.

On the Soil Conservation Reserve, Omarama, 100 acres of severely depleted and eroded North Western slopes of Omarama soil type were seeded at approximately 10 lb. per acre with a mixture of cocksfoot 6 lb., brown top ½ lb., white clover 1 lb., subterranean clover 2 lb., and yarrow ½ lb. per acre.

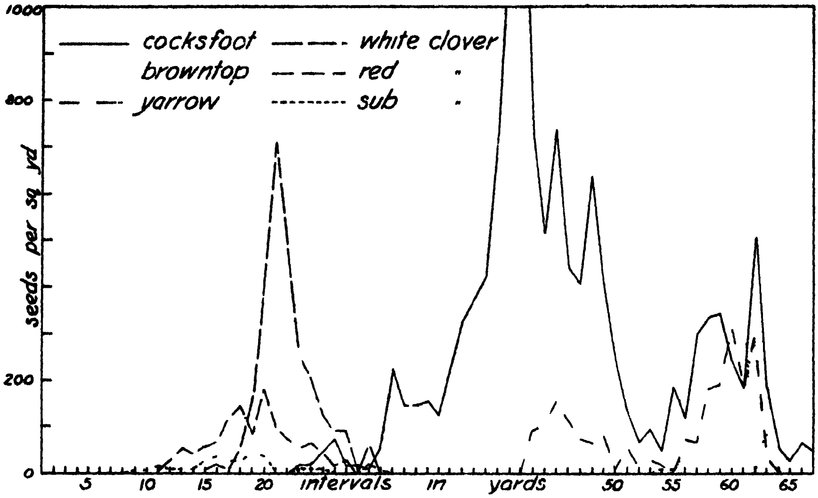


FIG 8 – Distribution of seeds released from a plane flying at 200 ft height on a calm day

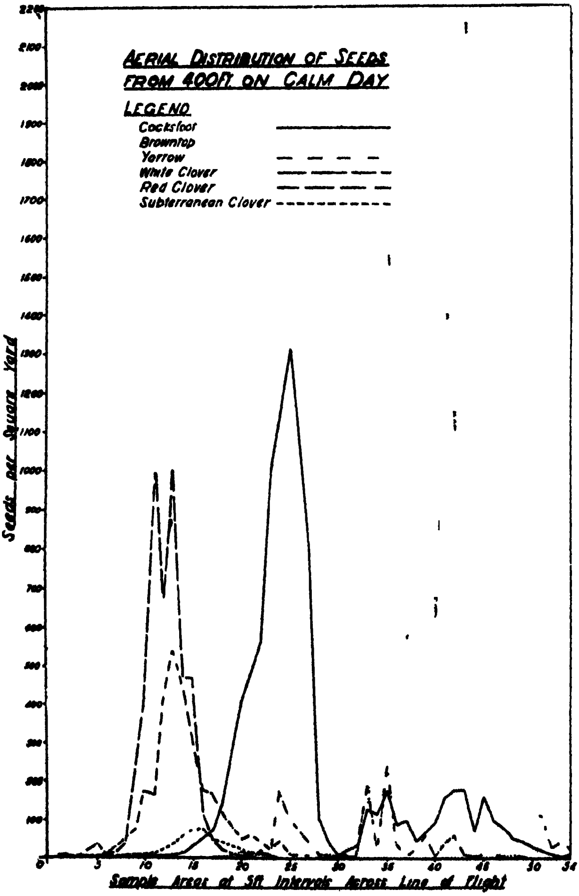


FIG 9 —Distribution from 400 ft. on a calm day

A temporary landing strip from which the machine operated was made on the adjacent flats by filling in some rabbit warrens, and the Army provided walkie-talkie equipment to provide communication between the observer on the hillside and the pilot when he landed to re-load.

Despite a wind of 20-25 miles per hour velocity the trial was carried out and a satisfactory ground distribution was obtained by flying on approximate contours working in parallel strips 2 chains apart from the base towards the top of the slope. The fact that the whole operation was carried out in approximately 1 hour indicates the possibilities of cheaply sowing very large areas.

"Campden" Awatere Valley

Arrangements were made to sow a 50 acre trial block on the property of Mr. I. Cameron who had successfully surface sown depleted pastures in the past. Ideal ground conditions were obtained at the close of the frost season (September 30th) when the loose cracked soil was still moist.

However, on this typical high country (3,000 ft.) with its steep slopes and deep valleys, upper wind conditions were found to be dangerous for flying, although the ground weather conditions were ideal. In the runs made it was possible to lap the seed sown on each run but the winnowing effect of the clovers from the grasses in falling resulted in separate strips of these being sown on the ground. This would, however, be of little practical consequence if a large area was sown in a steady wind. Reports from ground observers confirmed the satisfactory spread of the seed on the ground.

Gisborne Hill Country

An early trial was made with sowing one pound per acre each of white clover and lotus major on this well grassed but clover-deficient hill country. Mr. Madden, Assistant Director, Grasslands Division, undertook the measuring of distribution.

Plates filled with water were placed 5 paces apart in the valley, on the slope, and along the top of the ridge to measure the distribution. The seed collected in the plates indicated a fairly satisfactory distribution but considerable variation in the numbers of seeds collected in some plates illustrated the effect of wind in modifying the fall of the seeds. However, stock would play a big part in further distributing seeds produced by the established plants.

CONCLUSIONS

The aerial distribution trials have proved that fertilizer can be dropped from aircraft successfully within the desired limits of accuracy, provided the ballistics of the fertilizer are reasonably uniform, and the aeroplane has satisfactory performances under the conditions of terrain involved.

From the preliminary measurements made of the distribution of grass and clover seeds from beneath the fuselage of a single engined plane, the indications are that satisfactory distribution and control of the individual components of the mixture is not obtained owing to the varying ballistics of the seeds. To some extent the problem was solved by sowing across wind in the field trials where a satisfactory distribution of the larger seeds was reported on the ground. The necessary control of the seed would appear at this stage to involve pelleting or cementing seeds into larger aggregates for aerial distribution.

Investigation Necessary

These trials have brought into sharper focus the need for considerable investigation in four major allied fields if this work is to be developed effectively.

1. Aviation :

Further information is required on the ballistics of falling particles (pellets of fertilizer and seed), the effect of wind, suitable containers and mechanical means of discharge, flying techniques on varying terrain, the effect of meteorological conditions on the periods suitable for flying in various regions, ground and air control of topdressing operations and design of types of aircraft most suitable for seed and fertilizer distribution.

2. Materials :

Investigations are needed to improve the efficiency of materials from the distribution and agricultural aspects. These.involve concentration of the fertilizer and improvement of its chemical and physical characteristics to assure lack of corrosion of the container, freedom of flow and control of the material during operations. Variation in chemical content of fertilizers and in mixtures of seeds will probably be required to suit different regions. Further investigations are necessary into the release of seeds from various places on the aircraft and on cementing them into aggregates over which greater control may be obtained. The availability and cost both of fertilizer and seeds are of considerable importance.

3. Transport and Economics :

Thorough investigation is necessary to evaluate the costs of aerial and normal topdressing in order to define the areas best serviced by each technique. Availability of and supply of fertilizer to aerodromes is basic to costing which may best be done on a self-contained commercial basis when suitable equipment and methods have been evolved. When these questions have been determined the most suitable method of allocating the cost among those receiving benefit—the landowner, the Catchment Board, the Air Force and the State—could be investigated.

4 Agriculture :

Information should be obtained relative to the total areas requiring seeding and topdressing, their location, the type of country and general topography, the proportion of Crown and private property, the most suitable seeding and fertilizing period, the most effective management before and after fertilizing or seeding, and the most critical areas from soil conservation and flood control points of view.

With this information available the Council will be in a strong position to organize a service that will help to achieve its purpose of soil conservation and flood control combined with the maintenance of permanent production from the land.

APPENDIX

Since these trials were commenced farmers have taken an active interest in aerial seed sowing and have contracted with Mr. T. J. Lucas, Managing Director of Southern Scenic Airtrips Ltd. to sow portions of their properties as follows :—

Mr. Scaife, Wanaka—1,500 lb. of seed was sown at the rate of 10 lb. per acre (approx.) at a cost of £10 for the aerial distribution.

Mr. Strain, Lake Hayes—1,800 lb. of seed was sown in 2 hours at the rate of 10 lb. per acre and at a cost of £10 : 10 : 0 for distribution.

Messrs. Saunders and Nelson, Crown Terrace—2,000 lb. of seed was sown in 2 hours at the rate of 10 lb. (approx.) per acre at a cost of £10 for distribution.

Wintersloe Run, Methven, a contract has been undertaken to sow 5,000 acres with a 10 lb. mixture of cocksfoot and white clover at an approximate cost of £166 for distribution.

ACKNOWLEDGMENT

The willing co-operation and enthusiasm of Departmental Offiecrs made these preliminary trials possible, and to the following departments the Soil Conservation and Rivers Control Council is indebted—Royal New Zealand Air Force, Department of Agriculture, Aerodromes Services, Ministry of Works, and Department of Scientific and Industrial Research.

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REVIEW

THE USE AND MISUSE OF SHRUBS AND TREES AS FODDER

IMPERIAL AGRICULTURAL BUREAU JOINT PUBLICATION No. 10 (1947)

pp 231 + XXXV, Price 9/- (sterling).

The importance of this subject, chosen for a joint publication by three of the Imperial Agricultural Bureaux, has been expressed adequately in the opening sentence: " It is a humbling fact for grass pasture experts to realise that probably more animals feed on shrubs and trees, or on associations in which shrubs and trees play an important part, than on true grass or grass-legume pastures, short and tall grass ranges and steppes ". As far as is known it is the first attempt to cover the subject comprehensively. For this reason it is necessarily sketchy but has served to draw attention to the lack of precise knowledge on the subject and to the paucity of investigation and research proceeding over the vast areas concerned. What was originally intended to be a compilation of " uses " has turned out to be equally a compilation of " misuses ", for the grazing of these areas is usually uncontrolled and closely connected with erosion problems and in places with forest management problems.

The main body of the publication consists of a series of contributions by local workers in many countries. Owing to lack of contributors from

Africa (other than the Union), India and Ceylon and the Mediterranean Lands, Near and Middle East these have been written up by R. O. Whyte, the Director of the Bureau of Pastures and Field Crops. Contributions cover Australia and its several states, New Zealand, South Africa, U.S.S.R., United States of America and Canada. An omission would seem to be some of the northern European countries other than Russia. Much stock is for instance depastured in the forest areas of Scandinavia. A section of Latin America has been omitted because the subject has been reviewed in Bulletin 36 of the Imperial Bureau of Pastures and Field Crops, " The Grasslands of Latin America ".

A chapter on the chemical composition and digestibility of fodder shrubs is included. This consists of a compiled list giving the results of all analyses made of these plants together with a reference to the original work. The publication is well rounded off with indices of authors and investigations, plant orders, genera and species, a key to common plant names, a geographical index, addresses of contributors and seventy illustrations. Apart from its value to workers interested directly in the subject the publication contains a wealth of information on plant geography of arid areas.

A. L. P.

AN INHERITED STRAW WEAKNESS IN WHEAT

By S. W. BOYCE, Wheat Research Institute, Christchurch, New Zealand*

(*Received for publication, 1st September, 1947*)

Summary

Evidence is given for the presence of a single gene causing pronounced weakness of straw in a *Triticum vulgare* cross.

INTRODUCTION

MECHANICAL breakage of straw in wheat frequently lowers the number of ears harvested and it is generally observed that the loss due to straw break is more serious in some varieties than in others. However, while straw weakness is selected against in economic breeding work, this character has not been studied genetically. The following analysis of an apparent single gene segregation for straw weakness is therefore published, with some reservations as to the reliability of the data which were recorded for a different purpose.

MATERIALS AND METHOD

The inheritance of yield and its components was being studied in a *Triticum vulgare* cross between the lines S 1556(P_1) and S 357(P_2). In recording number of ears per plant (e), the number of mature straws from which the ears had been lost by breakage (e_b) was recorded for each plant, for the purpose of correcting total yields in the final analysis. These breakages may have occurred while the crop was standing in the field, during harvest, or in the final handling of the plants, and no attempt could be made to determine the cause of breakage in any plant. It was clear however, that one parent line suffered a much greater amount of damage than the other, the F_1, F_2 and F_3 being intermediate in this respect.

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* Now Botany Division, Department of Scientific and Industrial Research, New Zealand.

The design of the experiment precludes the possibility of experimental bias ; it was sown in eight beds, each bed being composed of :—

Generation.	Number of Plots.	Number of Plants per Plot
P_1 and P_2	11	25
F_1	1	25
F_2	1	50
F_3	18	50

Within each bed parent plots, chosen at random, alternated with groups of two hybrid plots, these also being randomized. Plots were harvested in the order in which they were grown, and handled in approximately that order in recording e and e_b.

The assumption of a single gene difference between P_1 and P_2 is tested in two ways : (1) The whole of the data is used as it was recorded, F_3 plot means being used as estimates of F_2 plant means, and compared with theoretical F_2 values based on P_1, P_2 and F_1 plot means. (2) Samples of equal numbers of plants have been drawn at random from each generation, P_1, P_2 and F_1 distributions being used to test expected F_2 and F_3 plantwise distributions.

RESULTS

In Table I the number of broken straws is expressed as the percentage of total ears within each bed, and over all beds.

TABLE I. SUMMARY OF PERCENTAGE STRAW-BREAK PER BED

Generation.	Percentage straw-break per bed								Per cent. straw-break over all beds
	1	2	3	4	5	6	7	8	
P_1	14.4	8.6	5.7	27.6	17.4	18.1	22.9	17.2	15.9
P_2	3.7	0.6	2.2	2.9	4.6	3.6	1.9	2.9	2.8
F_1	12.8	3.4	2.2	4.6	9.6	0.6	7.9	14.0	6.8
F_2	4.5	6.6	5.0	5.6	36.2	8.0	2.2	7.1	8.4
F_3	7.7	3.3	2.9	7.8	6.1	5.6	10.1	9.2	6.9

From these figures it is apparent that the F_1, F_2 and F_3 are approximately intermediate in value, but approach P_2 rather than P_1. This may be due to a slight degree of dominance for strong straw, and at the same time, to geometric gene differences. In addition there is a marked correlation between mean and standard deviation of the parents and F_1:—

	P_1	P_2	F_1
Mean	15.9	2.8	6.8
Standard deviation ...	12.2	3.3	5.1

Since there is no genetic variation within parents and F_1, it is apparent that environmental, as well as genetic, variation is geometric. Therefore, geometrically increasing class intervals are used in studying the frequency distributions over all plots. The first class is based on one third of the standard deviation of P_1, including all values from 0-4 per cent., the second class covers from 5-16 per cent. and the third from 17 per cent. upwards. In Table II is shown the actual distribution of percentage breakage per plot, based on this classification.

TABLE II. FREQUENCY DISTRIBUTION OF PERCENTAGE STRAW-BREAK PER PLOT

Generation.	Numbers of plots in groups classified for percentage straw-break.			Total.
	0 - 4 Per cent.	5 - 16 Per cent.	17 Per cent. upwards.	
P_1	30	10	0	40
P_2	5	14	16	35
F_1	3	5	0	8
F_2	1	6	1	8
F_3	56	61	12	129

When dominance is slight, as in this case, the F_3 plot means may be taken as estimates of the genetic values of the F_2 plants which gave rise to them. Using the actual P_1, P_2 and F_1 distributions we can therefore construct theoretical F_2 distributions for comparison with the actual F_3 data. Assuming a single gene difference between P_1 and P_2, the F_2 distribution should be composed of $1P_1 : 2F_1 : 1P_2$. Since the actual F_3 is composed of 129 plots we may take 128 as a close approximation to the actual number; this would give a distribution of $32P_1 : 64F_1 : 32P_2$. Actual P_1, P_2 and F_1 distributions have been transformed into these proportions and summed, giving a theoretical distribution for comparison with the actual (Table III).

TABLE III. THEORETICAL RECONSTRUCTION OF F_3 PLOT MEAN PERCENTAGE e_b FREQUENCY DISTRIBUTION

Generation.	Numbers of plots in groups classified for percentage straw-break.			Total.
	0 - 4 Per cent.	5 - 16 Per cent.	17 Per cent. upwards.	
P_1	24.00	8.00	0.00	32
P_2	4.57	12.80	14.63	32
F_1	24.00	40.00	0.00	64
Theoretical F_3	52.57	60.80	14.63	128
Actual F_3	56.00	61.00	12.00	129
Difference	−3.43	−0.20	+2.63	1
χ^2	0.22	0.00	0.47	0.69
				P = 0.7

The χ^2 value (0.69, P = 0.7) shows that there is no significant difference between the theoretical and actual distributions. The single gene assumption is therefore not disproved by this analysis.

In order to test the single gene assumption further, a random sample of 150 plants was drawn from each generation, e and e_b being summed for each sample (Table IV).

TABLE IV. DISTRIBUTION OF PLANTS CLASSIFIED ACCORDING TO NUMBER OF BREAKS PER PLANT

Generation.	Number of breaks per plant.					Total e_b	Total e
	0	1	2	3	4 - 8		
Number of plants in each class :— P_1	82	35	20	8	5	125	740
P_2	128	16	2	4	0	32	866
F_1	114	22	9	4	1	60	923
F_2	107	30	7	2	4	69	802
F_3	112	22	7	4	5	70	753

From these data, theoretical F_2 and F_3 plantwise distributions have been constructed, based on the equations $F_2 = 1P_1 + 2F_1 + 1P_2$ and $F_3 = 3P_1 + 2F_1 + 3P_2$. Agreement is again fairly good:—

Class.	0	1	2	3	4-8
F_2 Actual Distribution	107	30	7	2	4
*F_2 Theoretical Distribution	109	24	10	5	2
χ^2	0.04	1.50	0.90	1.80	2.00
Total $\chi^2 = 6.24$, P = .2					

Class.	0	1	2	3	4-8
F_3 Actual Distribution	112	22	7	4	5
*F_3 Theoretical Distribution	107	24	11	6	2
χ^2	0.25	0.17	1.45	0.67	4.50
Total $\chi^2 = 7.02$, P = .15					

* For method of construction of theoretical distributions see Table III

The above comparison is based on equal numbers of plants. Since the samples did not have equal *e* values, a final comparison has been made after adjusting all lines to the same *e* (740) as P_1, using the equations

$$F_2 e_b = 1P_1 e_b + 2F_1 e_b + 1P_2 e_b, \text{ and}$$
$$F_3 e_b = 3P_1 e_b + 2F_1 e_b + 3P_2 e_b:$$

	P_1	P_2	F_1	F_2	F_3
e	740	740	740	740	740
Actual e_b	125	27	42	64	69
Expected e_b	125	27	42	59	68

Agreement is again quite good.

It is therefore concluded that a single gene or closely linked gene complex determines the difference in strength of straw, between S357 and S1556.

NOTE ON THE ESTIMATION OF BACTERIAL POPULATIONS

By P. B. HUTCHINSON, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

IN the course of work involving large numbers of approximate population determinations, a simple meter which uses cultural opacity as a criterion proved invaluable. If approximate estimates are sufficient, determination by relative opacity has several advantages over dilution-plate and microscopic-techniques; time and labour are reduced to a minimum; there is no loss in volume or risk of contamination in repeated estimations of the same culture; both live and dead organisms are included in the estimation.

The apparatus (Fig. 1) consists of a lamp-house which directs light through the sample to be estimated. An adjustable diaphragm is located between the light-source and the sample tube. The tube-holder is slotted and allows a narrow beam of light to pass through the tube to an ordinary photo-electric exposure meter. This gives a direct reading of the light transmitted by the sample.

The culture tubes are previously selected to conform to uniform diameter and wall thicknesses, thus eliminating errors which would arise due to varying optical properties of different-sized tubes. To correct errors due to change in light-intensity a constant voltage source is used. The diaphragm allows standardization of the instrument against check tubes in each experimental series.

The instrument is calibrated empirically against cultures of which populations have been previously determined microscopically It is thus possible to compute the curve correlating population with relative cultural opacity. Recalibration is advisable for each new batch of medium used; errors arising from varying light-scatter and wave-length are thus minimized.

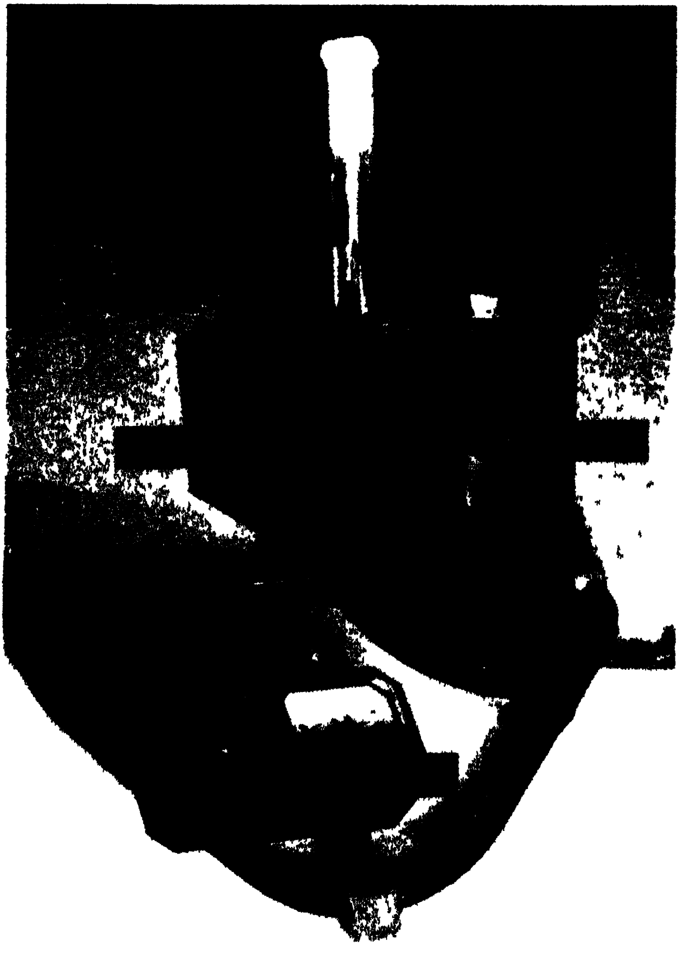

FIG. 1

LIGHT-LEAF-SPOT OF BRASSICAS

By H. C. SMITH, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 1st June, 1948*)

Summary

1. Light-leaf-spot of brassicas caused by *Gloeosporium concentricum* is widely distributed on cauliflowers in market gardening areas near Auckland city.
2. Degree of infection varied from 0 to 100 per cent. in different crops.
3. Characteristic symptoms are leaf distortion and the presence of superficial white spore masses on leaves
4. The disease is of little economic importance, its effect on yield and quality being slight
5. There is considerable variation in varietal susceptibility, a number of late maturing cauliflowers and one cabbage variety being immune.
6. The causal fungus grows readily on a variety of media but growth is slow.
7. Infection was secured in both field and cold frame by artificial inoculation of plants, symptoms appearing after 20 days.

INTRODUCTION

LIGHT-LEAF-SPOT of brassicas is widespread in England where it has been recorded on broccoli*, cauliflowers and spring cabbage (Moore, 1943). The fungus causing this disease was recorded in New Zealand by Cunningham (1944) under the original name *Cylindrosporium concentricum* Grev. ex Fr. Use of the combination *Gloeosporium concentricum* (Grev.) Berk. and Br. has been recommended by the British Mycological Society (Moore, 1939), since retention of the species under *Cylindrosporium* would so alter the concept of this genus that changes in 155 specific names would become necessary.

INCIDENCE

Light leaf-spot is widely distributed on cauliflowers in all market gardening districts in the vicinity of Auckland. Occasionally cabbage plants also are attacked, but in three cabbage crops at Pukekohe no infected plants were seen.

On cauliflowers incidence of light leaf-spot varied from nil to 100 per cent. In most crops where the disease was present every plant was infected but there were wide differences in the amount of leaf area affected. To secure information concerning severity of attack the approximate percentage of leaf area diseased was used in assessing degree of infection. For the purpose of comparison three grades were used: (a) Nil—no infection, (b) moderate—0-50 per cent. leaf area diseased, (c) severe—50-100 per cent. leaf area diseased. Degrees of infection in the crops inspected are shown in Table I.

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* In this paper broccoli varieties are included as late maturing cauliflowers.

TABLE I. DEGREE OF LIGHT-LEAF-SPOT INFECTION ON CAULIFLOWERS IN THE AUCKLAND AREA

District.	Season.	Number of Crops infected. (percentage leaf area infected)			
		No Infection.	0-50 per cent.	50-100 per cent	Total No Crops.
Mangere	June-July	9	4	1	14
Pukekohe	June-July	3	2	0	5
Avondale	July-August	0	1	0	1
					20

In no case did the disease appear to be of economic importance. The single instance of severe infection was a poorly grown crop of an early maturing variety grown out of season. The crop at Avondale showing 50 per cent. infection was again recorded after marketable cauliflowers had been cut, to measure the losses caused by this disease. One tenth of the cauliflowers had been rejected for various reasons, e.g. small size, and open curd, but only 20 per cent. of these showed severe infection with light leaf-spot. Thus the number rejected because of this disease could not have amounted to more than 2 per cent. of the total crop.

SYMPTOMS

The disease may be found at any time of the year, but appears to develop most readily under cool rainy conditions and on slow growing plants. The main symptoms in the field are distortion of leaves, and a white film of spores. Lesions occur independently on both leaf surfaces and on stems as circular or irregular light coloured patches surrounded by concentric circles of small white spore masses (Fig. 1). The most characteristic symptom of severe infection is malformation of leaves (Fig. 2). Size of lesions varies from about half an inch to three inches or more in diameter. Infection of the whole leaf surface has been obtained by artificial inoculation, but is rare in the field. Spores are pushed out in minute white tendrils on to the leaf surface. These tendrils are flattened by dew or rain to form a white film resembling spray residue. Heavy rain however, quickly washes away the spores. Irregularly shaped black spots may appear in the positions previously occupied by spore tendrils, but these are superficial only. A later symptom of severe infection is a silvering of the leaf surface, caused by lifting of the cuticle.

GROWTH IN CULTURE

The fungus was isolated by transferring spores from a diseased cauliflower leaf to cabbage leaf extract agar (200 g. cabbage leaves, plus 200 ml. water, plus 8 g. agar agar). Subsequently the fungus was transferred to potato dextrose agar, prune agar, malt agar, and Czapek's synthetic media. It spored freely except on Czapek's media, on which it remained sterile. Growth was slow on all media. Optimum temperature for growth in culture was found to be 20°c.

MORPHOLOGY

Development of the fungus in the leaf is superficial, as hyphae are found only within the cuticle. Spores are budded off from thickened hyphae which form an acervulus also in the cuticle (Figs. 3 and 4). As spores increase in number pressure ruptures the cuticle and they are forced through the opening forming a tendril. Spores from cauliflower leaves have an average size of 10.0μ × 2.4μ and a range of 6.5 to 14μ × 2 to 3μ. These dimensions are in agreement with measurements recorded by Thomson (1935) Spores are allantoid, often slightly curved, single celled (or occasionally one-septate) and have rounded ends. They germinate with a single polar germ tube, usually forming a septum across the spore somewhere between the centre and the germ tube end

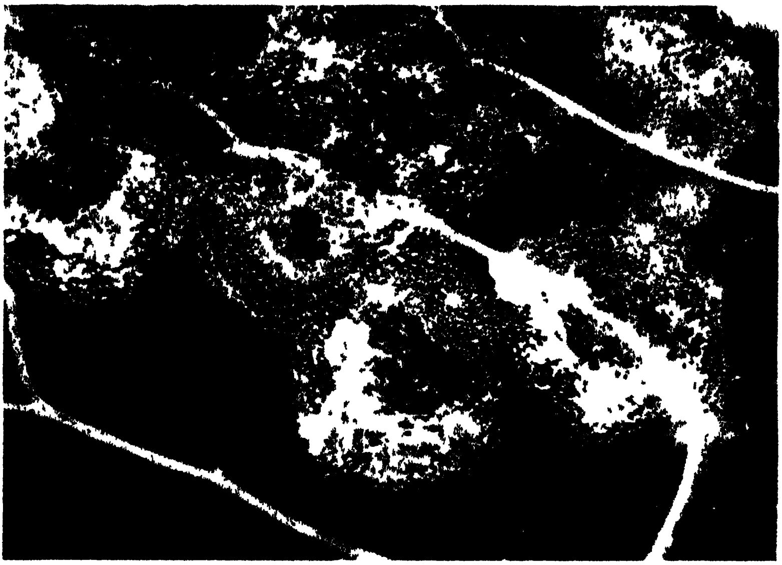

FIG. 1 Typical lesion on cauliflower leaf showing concentric spore masses and white film of spores X2

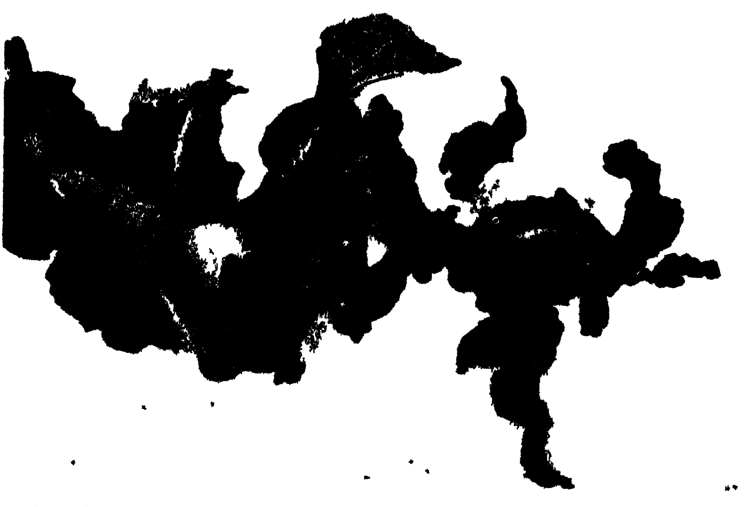

FIG. 2. Left · healthy cauliflower Right . severely infected cauliflower with distorted leaves

INOCULATIONS

Cauliflower seedlings of Phenomenal Early variety were grown in April and planted both in the field and in the cold frame. They were inoculated at fortnightly intervals with spores firstly from diseased leaves and later from culture. All plants became infected 20 days after inoculation both in the field and in the cold frame

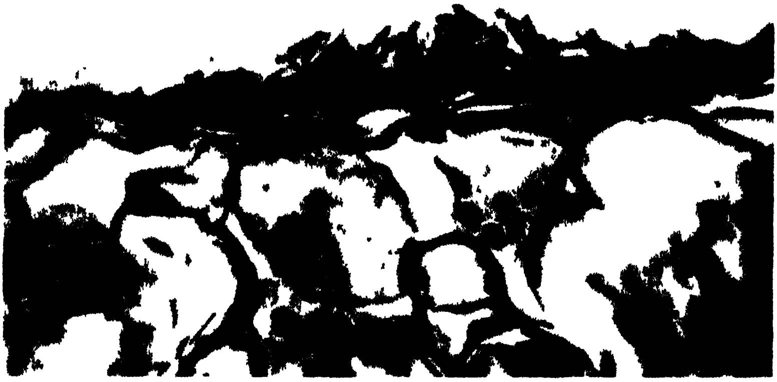

FIG 3 Transverse section of an acervulus on a cauliflower leaf, showing hyphae in the cuticle and spores X500

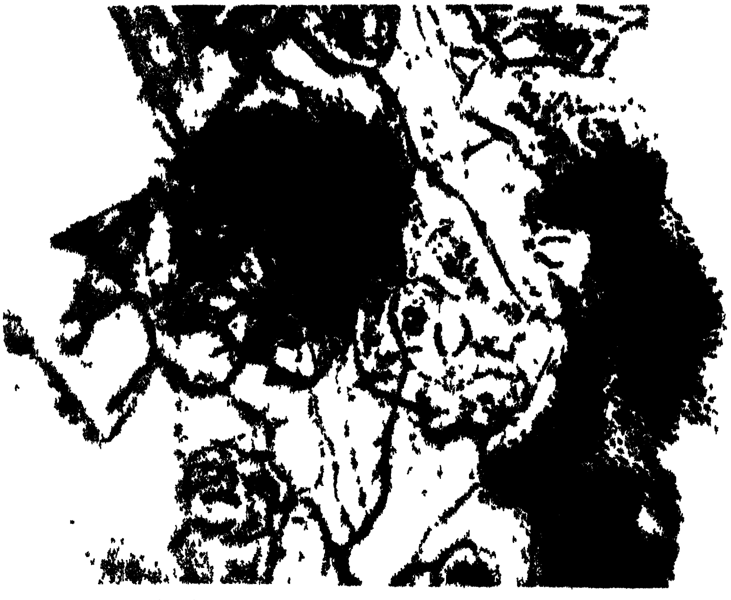

FIG 4.—Surface view of epidermis showing hyphae forming two acervuli X250

RESISTANCE

An unnamed cauliflower variety inoculated under the same conditions as Phenomenal Early failed to develop infection. This indicated the existence of resistant varieties, and suggested the necessity of classifying them. Twenty-three varieties of cauliflower and four of cabbage were tested for resistance to light-leaf-spot. Plants were raised in a cold frame, each variety being represented by one 6" pot containing 24 plants. When six inches tall they were inoculated by means of a hand atomiser with a water suspension of spores from potato dextrose agar culture. After inoculation plants were placed in a cabinet for 24 hours at 18 C and 100 per cent. relative humidity. They were then replaced in the cold frame for three weeks. At the end of this period spots were sufficiently well developed for differences in resistance to be determined. Two grades of infection were recorded; viz. *Moderately susceptible* with less than half the leaves infected and *Very susceptible* with more than half the leaves infected. Results are shown in Table II.

TABLE II. VARIETAL RESISTANCE OF CAULIFLOWER AND CABBAGE SEEDLINGS TO *G. concentricum*

Cauliflower, Early Maturing	Immune	Nil		
	Moderately Susceptible	Nil		
	Very Susceptible	All the Year Round Early London Late Metropole Novo Phenomenal Early	Phenomenal Maincrop Snowball A Snowball Southern Cross Super Snowball	Veitches Autumn Giant Veitches Self Protecting Walcheren
Cauliflower, Late Maturing (Broccoli)	Immune	Late Queen Penzance Roscoff No 1	Roscoff No 4 Satisfactory St. George	Snows Winter White
	Moderately Susceptible	Late White		
	Very Susceptible	Early White	Speeds Phenomenal	
Cabbage	Immune	Enfield Market		
	Moderately Susceptible	Early Market	Golden Acre	Jersey of Wakefield
	Very Susceptible	Nil		

To test resistance under field conditions inoculated cauliflower plants of eleven varieties were grown in the field during the summer months of December, January and February. Results were similar to those obtained in pots; the different varieties showing the same relative resistance.

CONTROL

There is no record of light-leaf-spot becoming serious enough to warrant use of control measures. Immune varieties of cauliflowers and cabbages have been found and although the former are all late maturing varieties, these could be grown if the disease should assume economic importance.

ACKNOWLEDGMENT

The writer wishes to acknowledge the assistance given by Messrs. S. O. Gillard and T. C. R. Dawe, Horticulture Division, Department of Agriculture. Thanks are also due to the Agronomy Division, Department of Scientific and Industrial Research, for supplying seed.

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SULPHUR DIOXIDE AND STORAGE LIFE OF DEHYDRATED APPLES.

By J. L. MANGAN, Plant Chemistry Laboratory, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication, 30th April, 1948*)

Summary

The retention of sulphur dioxide and storage life of dehydrated apples packed in standard cartons depends on the variety and storage temperature

A cellophane wrap was found necessary for the effective retention of sulphur dioxide and rapid losses occur in packets of apples which have been partly used

The loss of sulphur dioxide is roughly proportional to the titratable acidity and recommendations have been made that the acid varieties Ballarat and Washington should be marketed first, followed by Granny Smith, Statesman, Jonathan and Dunns

INTRODUCTION

THE dehydration of apples in New Zealand, which developed during the war, has now been converted to normal commercial practice, the apple slices being placed on the market in ½ lb. cardboard cartons with a cellophane over-wrap. The present work was carried out to investigate the effects of variety, sulphur dioxide content and temperature on the storage life of dehydrated apples under these conditions, and in addition to determine whether the cellophane over-wrap improved the storage life of the product.

STORAGE AT ROOM TEMPERATURES AND AT 24°C.

The dried apples were obtained from the dehydration plant containing only a trace of sulphur dioxide. Prior to dehydration the apple slices had been treated with a sulphite dip to prevent browning during dehydration. The residual sulphur dioxide was determined and as in the commercial process, metabisulphite added to bring the sulphur dioxide content to the required levels. The varieties Jonathan, Dunns, Statesman, Washington, Ballarat and Granny Smith were obtained, and samples of each variety were brought to levels of 50, 100, 250, 500, 1,000 and 2,000 parts per million of sulphur dioxide. The ½ lb. samples were weighed out, sprinkled uniformly with the calculated weight of sodium metabisulphate, which was itself checked for purity, packed into cartons and sealed with cellophane (Sidac 300 M.S.T.). Six samples at each sulphur dioxide level were prepared for each variety, three for warm storage and three for room temperature storage. The warm store was a " Pinex " lined room fitted with a fan and maintained at 24°C. (75°F.) by thermostatic control. Room temperatures during the storage period were normally in the range 16°-20°C. but dropped considerably on frosty nights. Sulphur dioxide, colour formation and moisture content were determined on the samples during storage.

Sulphur dioxide was determined by the A.O.A.C. Monier-Williams method (1), and colour was measured by a colorimetric method as follows :—

10 g. of minced sample is soaked for 15 min. in 100 ml. of aqueous alcohol (2 alcohol : 1 water) and then blended in a Waring blendor. The macerate is filtered through fine glass wool and allowed to run until optically clear. A sample is then taken and the brown colour measured in a Coleman spectrophotometer using a wavelength of 425 mμ. The extinction value is a direct measure of the amount of browning which has occurred in the sample.

Moisture was determined by drying for six hours in high vacuum at 65°C.

It was intended to carry out two analyses on each packet, resealing with cellophane after the first determination. This procedure, however, was found to give consistently low results for the second analysis as is shown in the sulphur dioxide graphs for Jonathan and Dunns, and a corresponding abnormal increase in colour formation. It was therefore possible to carry out only one analysis per packet at two monthly intervals instead of the intended monthly analyses. This abnormal loss of sulphur dioxide could be due (1) to merely opening the packet or (2) to a more rapid loss of sulphur dioxide from a half filled packet than from a filled one. In a later experiment where a portion of a packet was packed tightly into a screwtopped jar, this loss was not observed and hence the second explanation is probably correct.

The figures for sulphur dioxide, colour and moisture content are given in Table I and for ease of comparison they are plotted in Figs. 1 to 12.

In general all the varieties stored at room temperatures kept remarkably well. By comparing extinction values with visual colour estimation it was found that an extinction value of .200 corresponds to a slight browning in the apples while a value of .250 corresponds to a definite brown tint, at which stage the appearance would be considered unattractive. To be on the safe side the end of the storage life was taken as the point where the colour graph passes through the extinction value of .200. It should be noted that the end of storage life was determined by the formation of colour in the dehydrated apple itself, and that cooking tests were not carried out to determine flavour and appearance after cooking. By this standard, all the varieties at all sulphur dioxide levels kept for at least three months at room temperatures. The colour graphs however varied greatly and were rising with different slopes. Thus in all the samples of Ballarat the colour graph reached the extinction value of .200 within the storage period. In the higher sulphur dioxide levels of Washington and Granny Smith, however, the storage life was not completed in this time and an approximate value for the storage life was obtained by extrapolating the final section of the colour graph to the extinction value of .200. In the case of Dunns, and to a lesser extent Jonathans, colour formation was so slow that no estimate could be made of the storage life. Table II gives the storage life in days for each variety at each sulphur dioxide level.

TABLE I STORAGE OF DEHYDRATED APPLES AT ROOM TEMPERATURES AND AT 24°C AT DIFFERENT SULPHUR DIOXIDE LEVELS

		A		B		C		D		E		F.		Moisture
Variety	Time (days)	SO_2 p p m	Colour	SO_2 p.p m	Colour	SO_2 p p m	Colour	SO_2 p p m	Colour	SO_2 p p m	Colour	SO_2 p p m	Colour	(per cent)
Jonathan (24°c)	0	80	153	180	153	280	153	530	153	1030	153	2030	153	19 2
	32	36	177	22	200	59	208	210		518	-	1270		-
	60	29	345	21	270	24	252	125	200	348	180	885	138	
	90	19	295	19	293	40	265	115	233	390		1015		
	123	-	400		400	19	323	64	310	294	220	796	190	11 2
	158	6	350	6	380	15	360	78	385	298	.225	908	160	11 3
Jonathan (Room Temp)	0	80	153	130	153	280	153	530	153	1030	153	2030	153	19 2
	41	24	180	22	166	113	165	232	145	640	157	1382	157	
	89	29	—	42		75		165		433		944		
	122	19	207	13	187	46	169	296	177	513	145	1088	145	15 8
	186	3	215	3	200	46	173	128	155	336	125	955	115	15 5
Dunns (24°c)	0	50	120	100	120	250	120	500	120	1000	120	2000	120	22
	34	35	200	35	200	57	168	110	126	539	105	1065	090	
	63	10	220	10	237	25	200	95	180	310		800		
	93	20	260	22	230	30	230	82	193	375	138	942	106	16 2
	153	3	300	0	305	22	280	41	220	198	166	555	130	12 7
Dunns (Room Temp)	0	50	120	100	120	250	120	500	120	1000	120	2000	120	22
	36	54	120	54	120	143	-	300		710		1524		
	64	12	—	16		93		207		480		1036		
	93	25	143	132	143	76	130	210	120	505	120	1216	120	20 2
	160	0	152	0	152	38	140	107	140	370	095	840	095	20 2
Statesman (24°c)	0			155	108	255	108	505	108	1005	108	2005	108	18 4
	35			34	174	66	165	257	137	626	152	1500	152	16 6
	93			16	175	40	162	146	138	480	138	1322	100	15 8
	156			40	250	34	230	86	215	375	155	1126	150	14 4
Statesman (Room Temp)	0			155	108	255	108	505	108	1005	108	2005	108	18 4
	37			83	140	120	145	320	120	625	120	1352	120	18 3
	93			46	120	78	108	230	102	598	105	1350	105	18 7
	157			30	200	55	160	214	140	542	120	1233	120	18 2
Washington (24°c)	0			116	100	266	100	516	100	1016	100	2016	100	
	36			11	220	32	190	155	.165	400	150	970	143	18 5
	92			6	330	13	310	43	220	270	177	632	-	17 1
	156			22	510	25	535	60	390	153	350	550	190	14 8
Washington (Room Temp)	0			116	100	266	100	516	100	1016	100	2016	100	
	37			35	162	73	123	258	.123	530		1270		20 1
	92			10	170	46	140	158	135	403	102	940	097	19 8
	157			6	220	54	220	102	200	304	145	820	140	18 9
Ballarat (24°c)	0	67	135	117	135	267	135	517	.135	1017	135	2017	135	16 9
	34	22	255	13	290	26	270	124	230	386	185	1183	170	17 0
	100	36	380	8	375	30	340	30	325	214	210	559	130	15 5
	150	30	640	10	650	40	620	34	.570	86	480	382	349	14 1
Ballarat (Room Temp)	0	67	135	117	.135	267	.135	517	135	1017	135	2017	135	16 9
	36	12	190	12	200	64	—	200	- -	462	153	1230	145	18 0
	101	22	190	12	200	25	.150	102	145	339	125	838	095	18 1
	153	10	400	6	380	22	.335	23	290	215	.230	635	200	17 8
Granny Smith (24°c)	0	30	122	80	122	230	122	480	122	980	.122	1980	122	10 5
	31	36	—	57		125	—	300		663	—	1336	-	12 0
	96	18	200	12	225	60	.185	246	.180	495	.150	1000	120	-
	157	22	370	27	320	82	.280	148	.245	546	210	942	200	11 [illegible]
Granny Smith (Room Temp.)	0	30	.122	80	122	230	.122	480	.122	980	.122	1080	122	10 5
	31	37	-	65	—	125		310	—	528	- -	1520		13 5
	116	30	.155	45	160	97	155	218	148	538	.125	958	.125	15.8
	158	21	.250	11	245	88	230	179	195	398	.180	1040	175	16 8

(The vertical columns A, B, C, are the SO_2 levels, 50, 100, 250, . p.p.m)

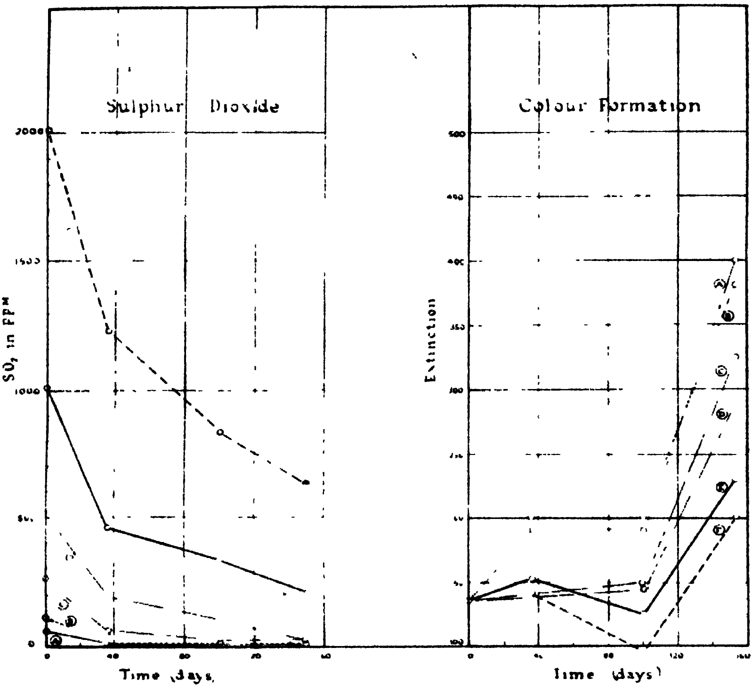
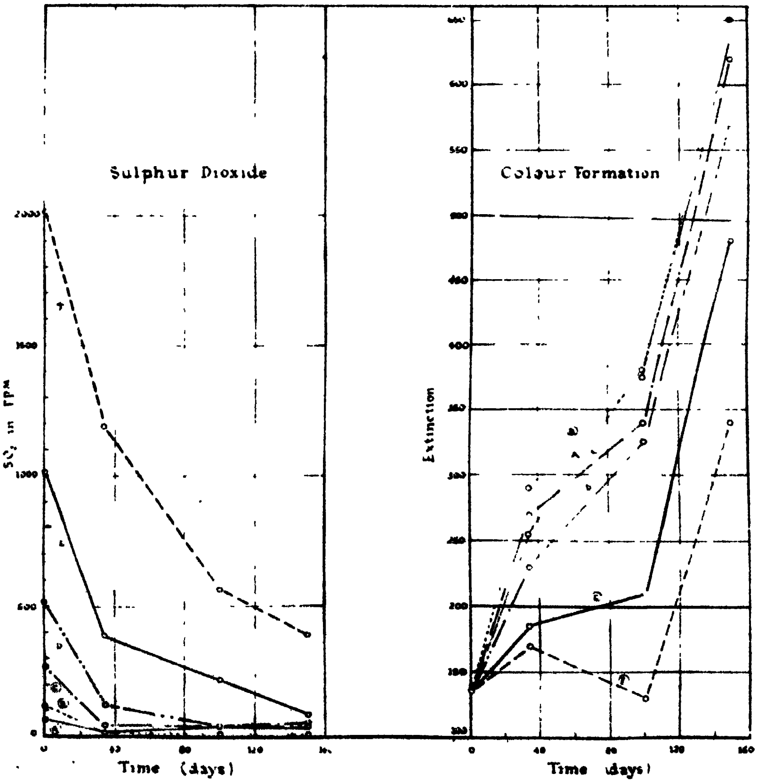


FIG. 1.—Storage of Ballarat apples at room temperatures



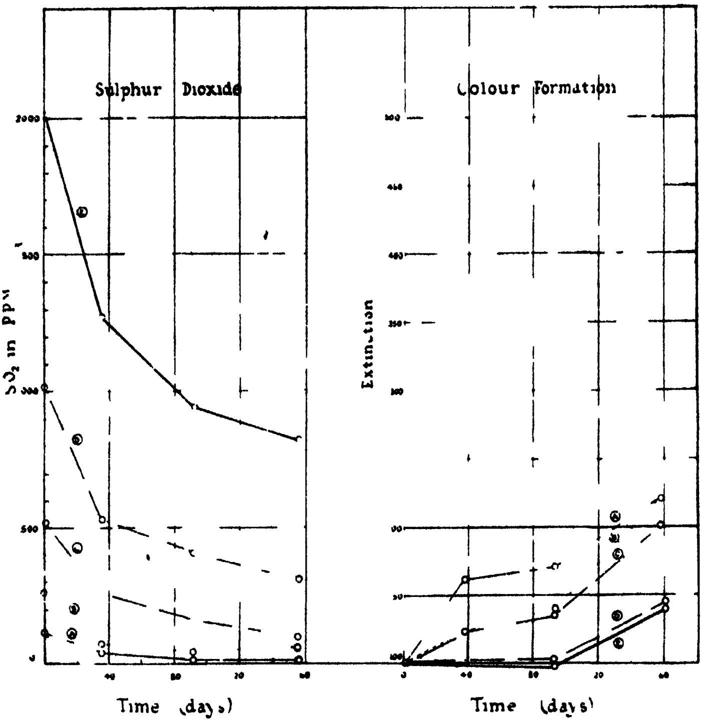


FIG 3 Storage of Washington apples at room temperatures

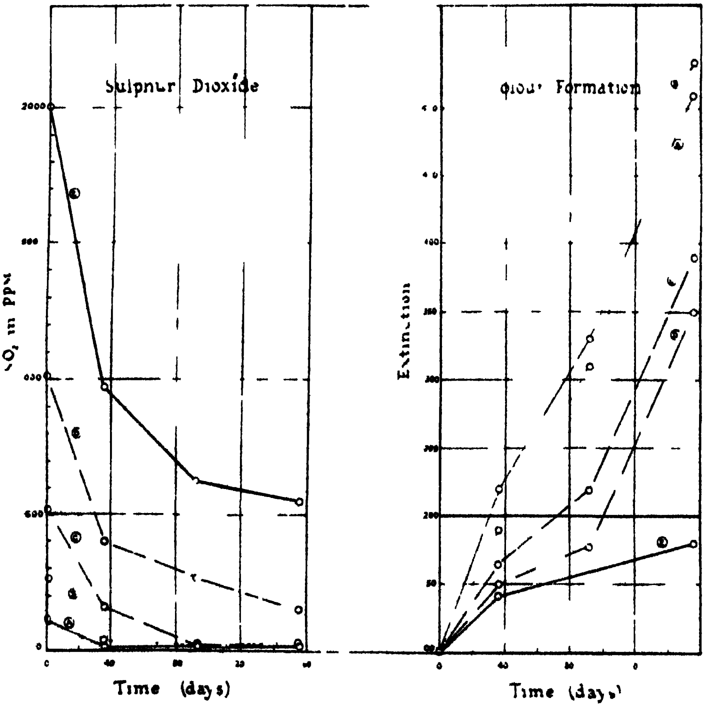


FIG 4 — Storage of Washington apples at 24°c

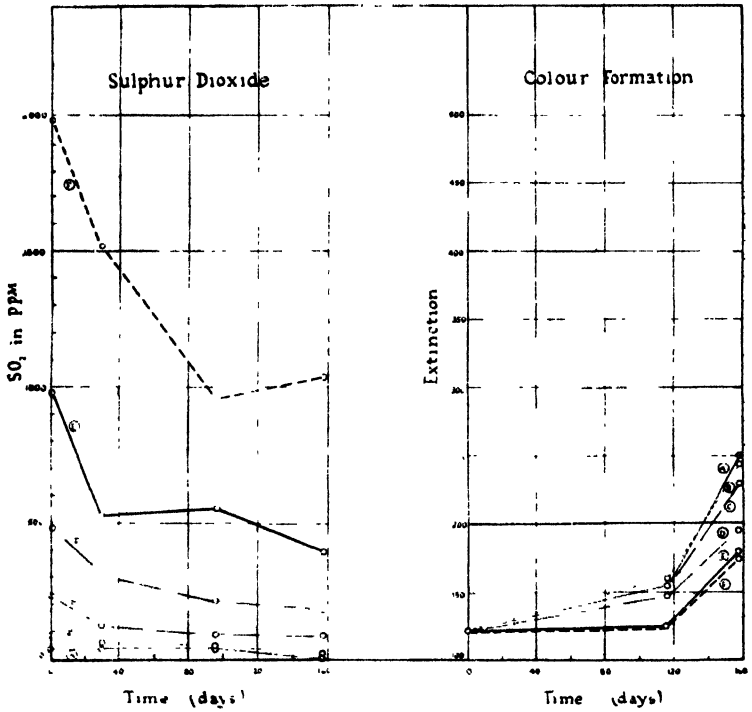


FIG. 5.—Storage of Granny Smith apples at room temperatures.

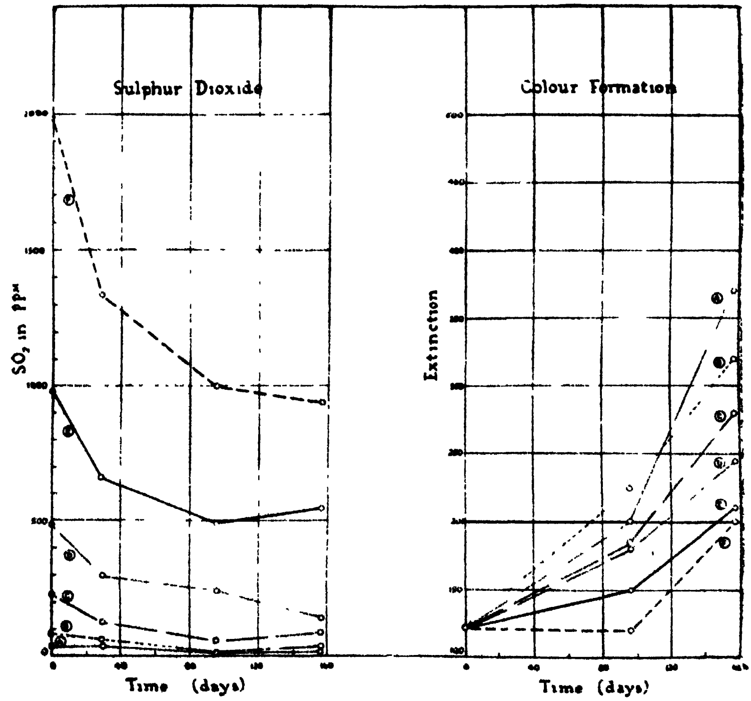


FIG. 6.—Storage of Granny Smith apples at 24°c.

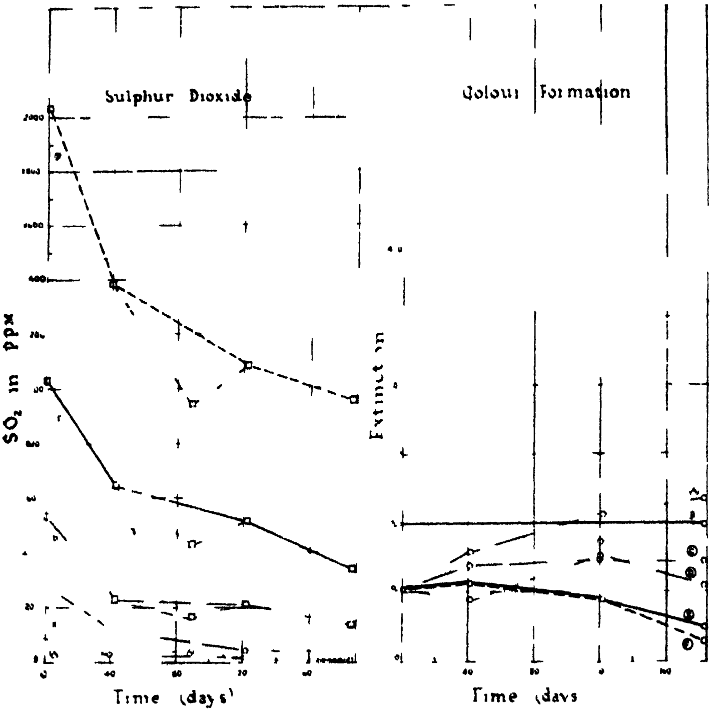


FIG 7 Storage of Jonathan apples at room temperatures

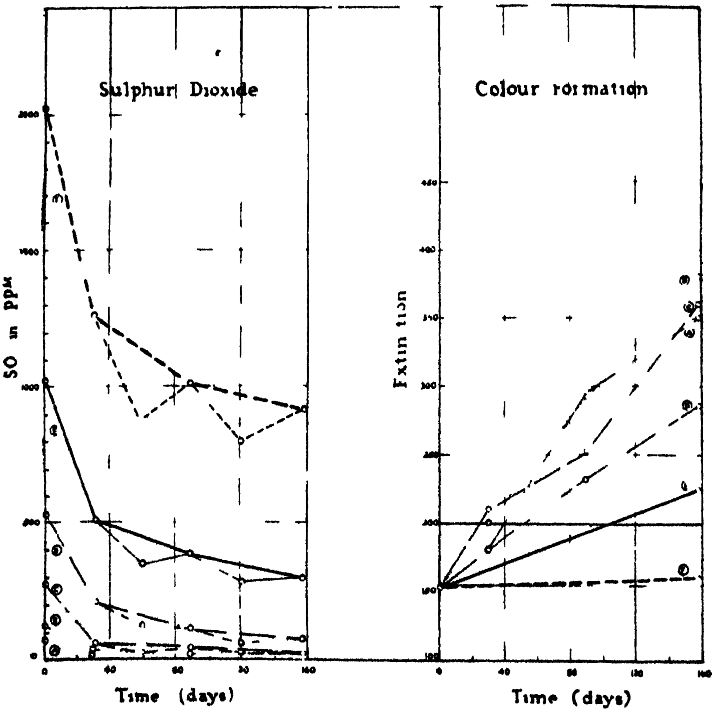


FIG 8—Storage of Jonathan apples at 24°C

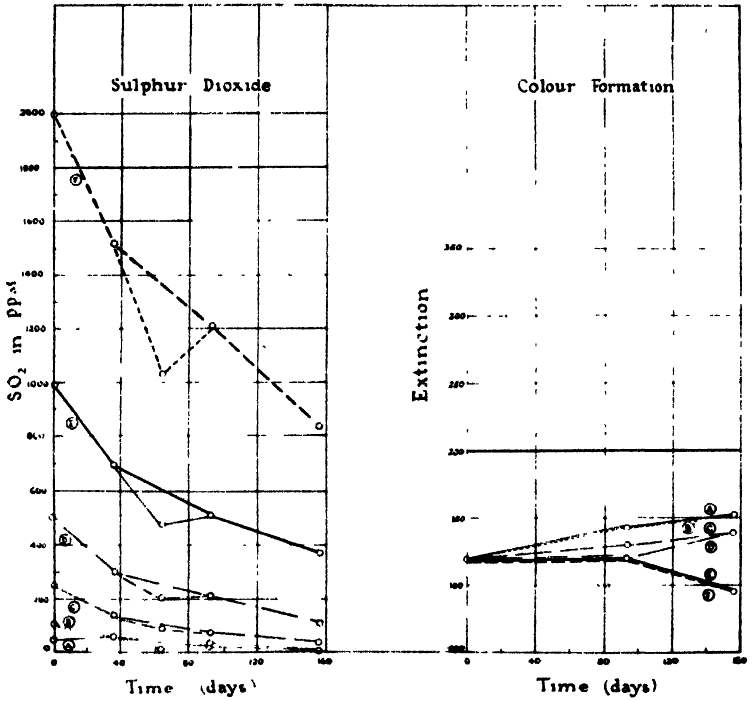


FIG. 9.—Storage of Dunn apples at room temperatures.

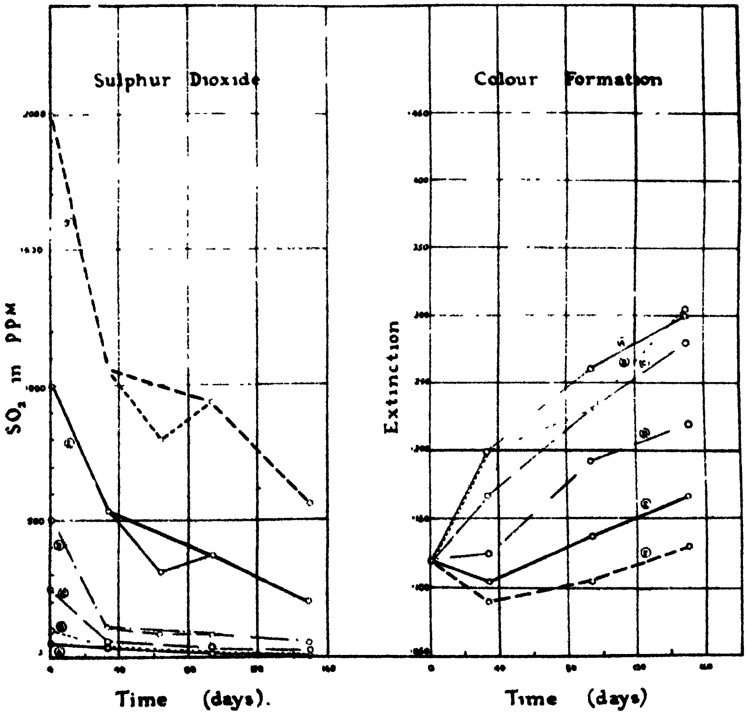


FIG. 10.—Storage of Dunn apples at 24°c.

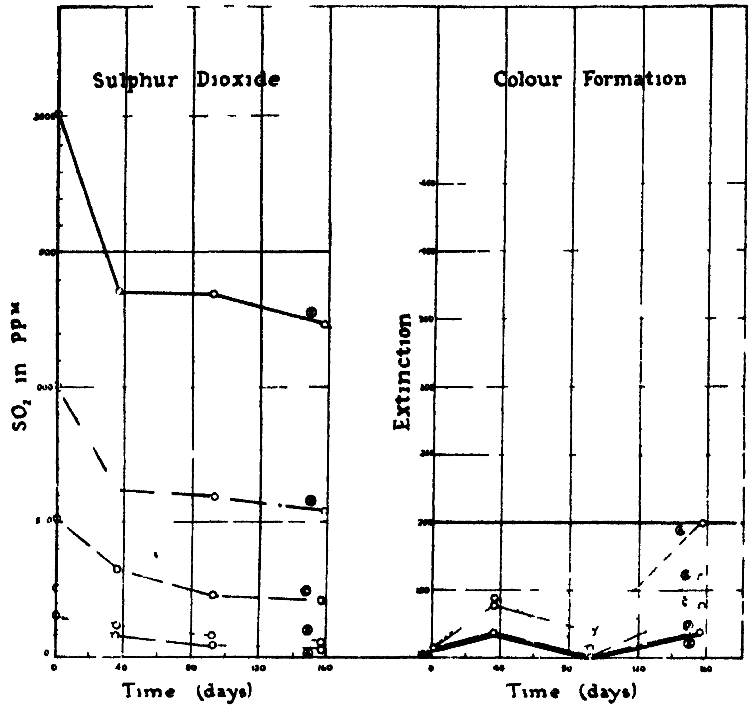


FIG 11.—Storage of Statesman apples at room temperatures

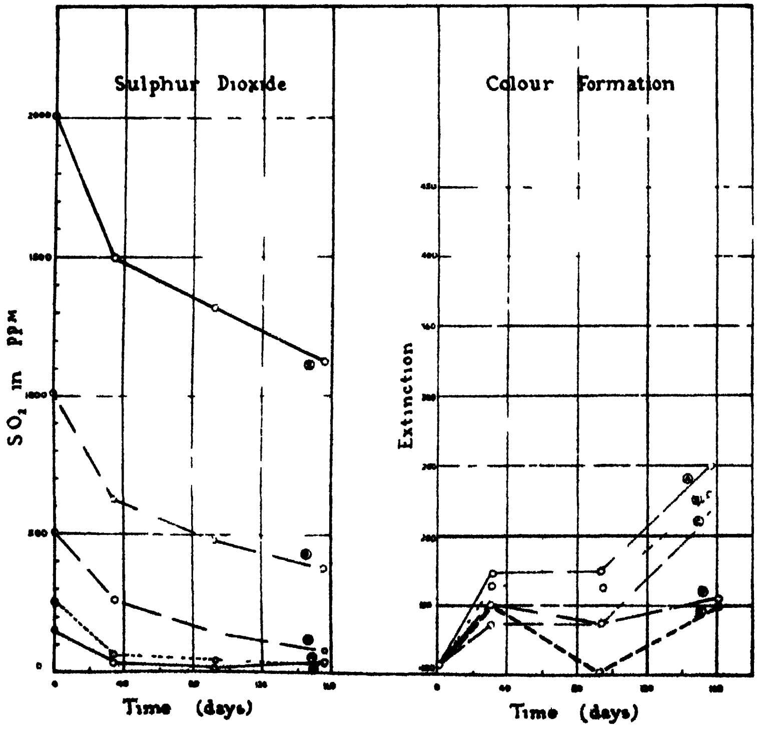


FIG. 12.—Storage of Statesman apples at 24°c.

TABLE II. STORAGE LIFE AT ROOM TEMPERATURES AT DIFFERENT SULPHUR DIOXIDE LEVELS

Variety.	50 p.p.m. SO_2.	100 p.p.m. SO_2.	250 p.p.m. SO_2.	500 p.p.m. SO_2.	1000 p.p.m. SO_2.	2000 p.p.m. SO_2
Ballarat	104 days	100 days	116 days	120 days	136 days	154 days
Washington	-- ,,	132 ,,	140 ,,	156 ,,	250* ,,	250* ,,
Granny Smith	136 ,,	136 ,,	142 ,,	162* ,,	165* ,,	180* ,,
Jonathan	96 ,,	176 ,,	v.l.	v.l.	v.l.	v.l.
Dunns	v.l.	v.l.	v.l.	v.l.	v.l.	v.l.
Statesman		156 ,,	202* ,,	260* ,,	v.l.	v.l.

NOTE: (1) Slight variations from these sulphur dioxide levels occur and can be seen in Table I.
(2) * Indicates figures determined by extrapolation.
(3) v.l. Indicates very long storage life.

Thus 500 parts per million of sulphur dioxide, which has been the recommended quantity, will keep Ballarats for four months, Washington five months, Granny Smith five and a half months, Statesman nine months and Jonathan and Dunns for a very considerable time at room temperatures. The addition of the maximum of 2,000 parts per million of sulphur dioxide to Ballarats however, only extends the life to five months and Granny Smith and Washington to an estimated six and eight months respectively.

The warm storage figures show the same effect in an exaggerated manner. On the warm storage figures the varieties can be classified into groups thus :-

A. *Ballarat and Washington.*

The colour graphs for these varieties are very steep and sulphur dioxide is lost very rapidly. By comparison of the graphs for storage at 24°c. and at room temperatures it is seen that the relatively small rise in temperature causes a much greater formation of colour and accelerates the loss of sulphur dioxide.

B. *Granny Smith, Jonathan and Dunns.*

The slope of the colour graphs in these varieties at 24°c. is moderate. Comparison of graphs shows that warm storage causes only a slight increase in the rate of loss of sulphur dioxide, but that colour formation is quite markedly increased.

C. *Statesman.*

The sulphur dioxide curves are not very different in warm storage and at room temperatures. The slope of the colour curves is small and increase in colour formation due to warm storage is not very great.

This grouping is in agreement with the titratable acidity figures which are given in Table III. These figures were not determined on the dehydrated apples as they came to hand since the pH was expected to be more important, but were obtained from fresh apples in 1945. Sturmer, a very important variety not included in these storage trials has a titratable acidity of 135, and from this it can be judged to lie between Granny Smith and Ballarat, with a short storage life probably approaching that of Ballarat and Washington. The pH seems to be of little significance.

TABLE III. TITRATABLE ACIDITY AND pH

Variety.	Millilitres 0.1N. NaOH/100 g. fresh tissue.	pH.
Washington	166	3.48
Ballarat	165	3.21
Granny Smith	79	3.54
Jonathan	77	3.47
Dunns	--	3.44
Statesman	64	3.90

The behaviour of Dunns is anomalous, since although at room temperature sulphur dioxide is lost more rapidly than in Statesman, colour formation is definitely less. In warm storage, however, it behaves normally. Sulphur dioxide is lost more rapidly than in Statesman while the rate of colour formation is so much greater that Dunns falls into the group with Jonathan and Granny Smith.

NOTE ON THE VALUE OF WARM STORAGE EXPERIMENTS

Generally speaking, warm storage of dehydrated apples at 24°c. gives a fair indication of the storage life to be expected in ordinary storage, in that the varieties can be classified into groups, but an exact relationship is not obvious. The " average slopes " of the colour graphs, i.e., the total vertical height divided by the corresponding horizontal distance, both measured in units of length, are given in Table IV.

TABLE IV. AVERAGE SLOPES OF THE COLOUR GRAPHS

SO_2 Level.	Ballarat.	Washington.	Granny Smith.	Jonathan.	Dunns.	Statesman
50 p.p.m.	2.74	--	1.28	1.17	0.99	-
100 ,,	2.69	2.21	1.03	1.06	0.96	0.70
250 ,,	2.59	2.10	0.82	1.01	0.85	0.60
500 ,,	2.32	1.49	0.44	0.68	0.53	0.52
1000 ,,	1.84	1.28	0.46	0.38	0.24	0.22
2000 ,,	1.09	0.41	0.41	0.05	0.05	0.20

Within the range 50 to 500 parts per million of sulphur dioxide these values appear to fall fairly well into the three groups mentioned above. Thus group A has slope 3.0 to 1.5, group B 1.5 to 0.5 and group C 0.75 to 0.5. This does not hold for samples containing 1,000 or 2,000 parts per million of sulphur dioxide, while group C is not sharply separated from group B. A longer storage period, however, would probably have corrected these irregularities. It is considered from these results that storage at a higher storage temperature, say 37°c., for a shorter period would yield a similar but more clear cut grouping, and that after correlating with ordinary storage figures a rapid warm storage experiment could be used to predict the storage life of an unknown at ordinary temperatures.

THE EFFECT OF CELLOPHANE WRAP ON THE RETENTION OF SULPHUR DIOXIDE

The dried apple slices are marketed in ½ lb. standard cartons with a cellophane over-wrap, and although this is partly to improve the appearance of the package it was considered that the cellophane would probably retard the loss by diffusion of sulphur dioxide. The material used was " Sidac ", grade 300 M.S.T., which is heat sealing and partly moisture-vapour proof. Six ½ lb. samples of Dunns were made to 500 parts per million of sulphur dioxide and packed in cartons, half of which were sealed in cellophane and the remainder left unwrapped. The samples were stored at room temperatures and sulphur dioxide determined at monthly intervals. The results are given in Table V., and it is seen that even at room temperatures the cellophane had a marked retarding effect on the loss of sulphur dioxide. Without the cellophane the apples lost 86 per cent. of their sulphur dioxide in the first month, after two months all sulphur dioxide had gone, and after three months the sample had begun to turn brown, the storage life probably being between three and four months. The wrapped samples on the other hand still retained 150 parts per million after three months, and from the results of the above storage trials a storage life of 9 months or more could be expected.

TABLE V. BARRIER EFFECT OF CELLOPHANE ON LOSS OF SULPHUR DIOXIDE

Time (days).	Cellophane Wrapped.		Unwrapped.	
	SO_2	Colour	SO_2.	Colour
0	500 p.p.m	White	500 p.p.m.	White
34	320 ,,	,,	70 ,,	,,
62	203 ,,	,,	10 ,,	,,
92	154 ,,	,,	10 ,,	Slight brown

The above results, which were obtained with the non-acid variety Dunns, would be even more striking in the case of acid varieties such as Ballarats or Sturmers.

LOSS OF SULPHUR DIOXIDE FROM A PARTLY USED PACKET OF DEHYDRATED APPLE SLICES

It was considered important to know for domestic purposes whether it was necessary to place any unused portion of a packet of apples in a closed container or whether it was satisfactory to leave it in the packet.

Three ½ lb. samples of Dunns were made to 500 parts per million of sulphur dioxide, packed in cartons, sealed with cellophane and left for one month at room temperatures to allow the sulphur dioxide to become evenly distributed. The packets were then opened, each was divided into two parts, one half being packed into a screw-topped jar (which was fairly tightly packed) and the other half was replaced in the packet which was closed but not wrapped with cellophane. Storage was continued at room temperatures and sulphur dioxide determined at monthly intervals. The results are expressed in Table VI

TABLE VI. LOSS OF SULPHUR DIOXIDE FROM AN OPENED PACKET OF APPLES

Time (days).	In screw-topped jar.	In cellophane wrapped packet.	In unwrapped packet.
0		500 p.p.m.	
34		320* ,,	
62	234 p.p.m.		62 p.p.m
92	190 ,,		20 ,,
126	172 ,,		3 ,,

(* Figure assumed from previous experiment.)

As was expected the portion left in the packet lost sulphur dioxide very rapidly, while that in the jar retained it somewhat better than the unopened cellophane wrapped samples of the previous experiment. As in the previous experiment the results would be accentuated in the case of acid varieties of apples.

RECOMMENDATIONS

The results obtained in the investigations justify the following recommendations.

(1) The minimum initial sulphur dioxide content required to keep dehydrated Statesman, Jonathan and Dunn apples until new season's fruit is available is 500 parts per million.

(2) The minimum sulphur dioxide level for other varieties is 1,000 parts per million, but even at this level the storage life of Ballarat, Granny Smith and probably Sturmer apples is not likely to exceed 6 months.

(3) The order of marketing the dehydrated product should be Ballarat, Washington, Granny Smith, Statesman, Jonathan and Dunns. Although dehydrated apples of the Sturmer variety were not included in these trials it is considered that these should be marketed about the same time as the Granny Smith product.

(4) It is recommended that cartons be over-wrapped with a grade of cellophane which is impermeable to water vapour and to sulphur dioxide.

(5) It is suggested that the directions on the cartons should include a recommendation to the effect that if a portion of the contents of a carton are to be kept for some time, this should be transferred to a screw-topped jar or other air-tight container of suitable size.

ACKNOWLEDGMENTS

This work was carried out for the Internal Marketing Division, and the general outline for the investigation was planned by Dr. B. W. Doak, Plant Chemistry Laboratory and Mr. L. W. Tiller, Fruit Research Officer. The writer wishes also to thank Dr. Doak for his interest in the work and for assistance in writing the manuscript. Thanks are also due to Mr. R. M. Greenwood for figures on titratable acidity of apples.

REFERENCES

(1) Methods of Analysis — A.O.A.C. (6th Edition) p. 533.

TESTS WITH D.D.T. AND GAMMEXANE ON THE LARVAE OF A DERMESTID BEETLE (*ATTAGENUS* SP.), A PEST IN SOME NEW ZEALAND WOOLLEN MILLS

By R. A. HARRISON, Assistant Entomologist, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 18th February, 1948*)

Summary

The larvae of a Dermestid beetle (*Attagenus* sp.) damages cops of spun yarn in some New Zealand woollen mills. Tests are described in which the toxicity of D.D.T. and Gammexane to these larvae is established.

It is shown that scoured wool impregnated with a solution of D.D.T. in acetone at rates of 2 per cent., 5 per cent. and 10 per cent. D.D.T. by weight of wool, caused mortalities of 28.1 per cent., 29.2 per cent. and 38.3 per cent. of larvae respectively after 18 days exposure and mortalities of 58.2 per cent., 81.7 per cent. and 82.5 per cent. respectively after 35 days exposure.

HARRISON—TESTS WITH D.D.T. AND GAMMEXANE ON THE LARVAE OF A DERMESTID BEETLE (*Attagenus* sp.), A PEST IN SOME NEW ZEALAND WOOLLEN MILLS

Treatment of scoured wool with an acetone solution of benzene hexachloride at the rate of 5 per cent. of the gamma isomer by weight of wool caused complete mortality of larvae within 18 days.

D.D.T. incorporated in a neatsfoot batching oil emulsion and applied to scoured wool at the rate of 0.57 per cent. D.D.T. by weight of wool gave an average mortality of 75.2 per cent. of larvae in one test of 17 days duration and 72.5 per cent. in a second test of 14 days duration.

Continued contact of larvae with D D.T. is necessary to secure adequate control. The nature of the D.D.T. deposits on the wool and their significance in relation to mortality is discussed.

LARVAE of a Dermestid beetle (*Attagenus* sp.)* commonly called the "Woolly Bear" cause considerable damage in some New Zealand woollen mills to cops of spun yarn in store. Damage inflicted is characteristic in that larvae eat their way towards the centre of the cops and in doing so, weaken or break numerous parts of the thread, rendering it unusable. In this paper are described experiments to determine if treatment of scoured wool with D.D.T. or Gammexane would protect wool against these larvae.

METHODS AND RESULTS

It was first necessary to determine if D.D.T. and Gammexane are toxic to these Dermestid larvae. Once this was established it was then necessary to ascertain if the toxicant could be incorporated in one of the mill processes. Preliminary tests were therefore undertaken with treatments of wool using acetone solutions of D.D.T. and Gammexane, followed by tests with D.D.T. in a batching oil.

Wool used in the experiments was scoured fleece. *Attagenus* sp. larvae collected from damaged cops in a store room several days before the tests were commenced, were fed on scoured wool until required for the experiments. The D.D.T. used contained 99-100 per cent. para para isomer, while crude benzene hexachloride containing 13 per cent. of the gamma isomer was the source of Gammexane. The batching oil was neatsfoot oil.

All experiments were conducted in the laboratory in glass petri dishes, 3 in. diameter and ⅝ in. deep. In each dish approximately 0.6 g. of wool was placed, this being of such a bulk as to fill the dish and allow the lid to fit tightly. Twenty larvae of uniform size were placed in each dish; small larvae were not used. The petri dish containing wool and larvae was then placed in a larger dish, which acted as a trap for larvae which might escape and the whole left undisturbed in the laboratory.

Tests with Acetone Solutions.

Acetone solutions were used on the wool as follows: Four samples of wool, each 10 g. were taken from the scoured fleece and 50 ml. lots of acetone containing 0.2, 0.5, and 1.0 g. D.D.T. and 3.86 g. of crude benzene hexachloride were added to give 2 per cent, 5 per cent. and 10 per cent. D.D.T. and 5 per cent. Gammexane by weight respectively. The volume of acetone was sufficient to wet the wool thoroughly without leaving any residue unabsorbed. The acetone was allowed to evaporate immediately after application of the solution, evaporation being hastened by use of a small blower. Portions of each sample were then placed in petri dishes.

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* Identified by the Commonwealth Institute of Entomology, London.

ERRATA

Vol. 29, No. 1, (*Sec. A*), " Analysis of New Zealand Greasy Wool Production, 1943-44 Season " : Page 23, Table III.

Fleece wool	546,011	68.60
should read	546,011	68.67

Eyeclips, brands, crushed days, and black wool	2,662	0.40
should read	2,662	0.33

Page 24, Table IV.

Carding	379,	983
should read	379,	938

Vol. 28, No. 4, (*Sec. A*), " The polarographic Estimation of Ascorbic Acid in Milk " : On page 269 under *Titration method*, fourth line ***should read*** " solution containing 0.1 mg. per millilitre."

On page 272, line 17, ***should read*** " cysteine step was found not to be affected."

Vol. 28, No. 3, (*Sec. A*), " Characteristics of Milk-ejection Curve of Normal Dairy Cows " : Page 203, Table II. The heading " M.S." should be headed " t ".

Page 205, last paragraph, line two. The hyphen between the words " one " and " Quarter " should be omitted.

During treatment the solution tended to accumulate in the denser parts of the wool mass and to drain to the bottom as evaporation proceeded, with the result that the deposit of insecticide on the fibres was heavier in these parts than elsewhere. An effort to overcome this defect by teasing out the wool before addition of the solution was only partially successful. Because of this unevenness of deposit and because the 0.6 g. portions of the wool actually placed in the petri dishes were taken from those parts of the 10 g. samples which did not contain an obviously heavier deposit, the percentage of insecticide on the tested wool was probably below the figure aimed at.

Six replications of treatments and checks were included in the tests. Checks comprised untreated wool and wool soaked in acetone alone.

Larvae were placed in each dish on 12th September, 1946, and mortality counts were taken on 30th September and 17th October. When mortalities were being recorded it was observed that some of the larger larvae had pupated during the test period. Pupae were included in the count as live larvae. Results are given in Tables I and II.

TABLE I. EFFECT ON LARVAE OF *Attagenus* SP. OF D.D.T./ACETONE AND GAMMEXANE/ACETONE APPLIED TO SCOURED WOOL. 18 DAYS EXPOSURE

	Check Wool only	Check Wool and Acetone only	2 per cent D.D.T.	5 per cent D.D.T.	10 per cent D.D.T.	5 per cent Gamma isomer
Mean percentage mortality	2.6	1.8	28.1	29.2	38.3	100
Mean equivalent angle	7.1°	4.5	31.4	32.2°	37.1	90.0°

Standard error 3.8°
Difference required for significance at 5 per cent. level 11.02

TABLE II. EFFECT ON LARVAE OF *Attagenus* SP. OF D.D.T./ACETONE APPLIED TO SCOURED WOOL. 35 DAYS EXPOSURE

	Check Wool only	Check. Wool and acetone only	2 per cent D.D.T.	5 per cent D.D.T.	10 per cent D.D.T.
Mean percentage mortality	4.6	6.9	58.2	81.7	82.5
Mean equivalent angle	9.8	13.2°	49.9°	65.1	66.8°

Standard error — 4.1°
Difference required for significance at 5 per cent. level 11.9

Tests with Batching Oil.

Tests using a neatsfoot oil emulsion containing D.D.T. were carried out after completion of the acetone solution trials. Neatsfoot oil is one of the common batching oils used in New Zealand mills and is frequently used in a formula which results in the wool containing approximately 6 per cent. of oil. In these tests a similar formula was used and the method of application to the wool was the same as that used in mills. The emulsion was prepared as follows:

2 ml. neatsfoot oil containing 8 per cent. w/v D.D.T.*
1 ml. emulsifier made by dissolving bar soap in water.
7 ml. water

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* This was a saturated solution of D.D.T.

This was heated to 120°F. and sprayed onto 1 oz. of wool by means of an atomizer. The treated wool contained 0.57 per cent. D.D.T. by weight. The wool was left undisturbed for several days to allow excess water to evaporate. Portions were then placed in petri dishes and larvae added. The experiment included six replications of treatment and checks, the latter consisting of scoured wool alone. Tests were commenced on 26th October, 1946, and mortality counts were made on 12th November. Results are recorded in Table III.

TABLE III. EFFECT ON LARVAE OF *Attagenus* SP. OF D.D.T./NEATSFOOT OIL EMULSION APPLIED TO SCOURED WOOL. 17 DAYS EXPOSURE

Replications.	Percentage Mortality.	
	Check	0.57 per cent. D.D.T
1	0	100
2	0	47.3
3	5.5	94.4
4	0	68.4
5	0	75.0
6	0	65.0
Mean	0.9	75.2

Tests with neatsfoot oil were repeated on 31st March, 1947, to check results of the previous experiment. On this occasion a test with wool treated with neatsfoot oil emulsion, but without D.D.T., was added. There were six replications of each treatment. Mortalities were recorded on 14th April, results being given in Table IV.

TABLE IV. EFFECT ON LARVAE OF *Attagenus* SP OF D.D.T./NEATSFOOT OIL EMULSION APPLIED TO SCOURED WOOL. 14 DAYS EXPOSURE

	Check Wool only	Check Wool and emulsion only.	0.57 per cent D.D.T
Mean percentage mortality	2.5	5.0	72.5
Mean equivalent angle	8.1°	12.6	61.6°

Standard error = 4.6°
Difference required for significance at 5 per cent level = 13.9°

After mortalities had been recorded all live larvae from the six dishes containing wool treated with D.D.T./neatsfoot oil emulsion were placed in another dish containing scoured wool only. Seven days later 18.2 per cent. of these larvae were dead and after 5½ months the mortality was 45.4 per cent.

DISCUSSION

The toxicity of D.D.T. to *Attagenus* sp. larvae has been established in all tests. Results of the acetone solution trials (Tables I and II) show that at the level of significance stated there was no significant difference between the three D.D.T. treatments after the 18 day period; after 35 days, however, a significant difference was apparent between the 2 per cent. and 5 per cent. and 2 per cent. and 10 per cent. treatments, but not between the 5 per cent. and 10 per cent. treatments.

The tests with Gammexane have shown that it is highly toxic to the larvae, much more so than is D.D.T. at the same or double the dosage of active insecticidal material (Table I). Under conditions of the tests, however, it is possible that this insecticide exerted some fumigant action which increased the kill. An objectionable feature of the use of crude benzene hexachloride was the fact that the wool was stained a dull red. As this would be undesirable in mills, tests with this material were not carried further.

An examination of the mean percentage mortalities in Tables I and II in relation to the duration of the tests shows that the action of D.D.T. on these larvae when it is applied to wool in an acetone solution is comparatively slow. The maximum kill of all treatments was 82.5 per cent. after 35 days. This apparent slowness may be due to the fact that, when applied with acetone, D.D.T. is deposited in the form of coarse crystalline masses which adhere loosely to the fibres. The physical nature of the deposit possibly has some bearing on the relative slowness of the toxic action, as D.D.T. in large crystal form has been shown to have no marked lethal effect on bed bugs (*Cimex lectularius* L.) when compared with D.D.T. deposited as minute crystals (Barnes, 1945).

Results from the tests using D.D.T. in neatsfoot oil emulsion (Tables III and IV) show that D.D.T. in this form gave a more rapid kill of larvae than when deposited from an acetone solution. This can be accounted for by the fact that the oil solution of D.D.T. which remains on the fibres after the water has evaporated from the emulsion does not volatilise and leave crystals of D.D.T. to any extent. Consequently the larvae, when working their way through the wool, become coated with the solution. D.D.T. is thus brought into intimate contact with the entire body surface and being in an oil solution is more readily absorbed through the integument than when present in crystalline form.

That oil when used alone did not have any toxic effect on larvae under the conditions of the tests is shown in Table IV where there is no significant difference between the two sets of checks.

Continued contact with D.D.T. appears necessary foi satisfactory results. It has been shown that after 14 days exposure to D.D.T. emulsion on wool, of larvae which were alive at the end of the period and which were transferred to wool uncontaminated with D.D.T., only 18.2 per cent. or 5 per cent. of the original total were dead after a further seven days. The amount of D.D.T. absorbed during the 14 days was not sufficient to cause death in all larvae as at 5½ months a percentage was still alive and these subsequently pupated and emerged as adults.

In the light of results obtained in these experiments, application of D.D.T. in neatsfoot oil emulsion offers a promising means of control of larvae in woollen mills. Further work is needed to determine whether D.D.T. in the oil will remain on the fibres in sufficient concentration to be toxic to larvae after the wool has been carded and spun into yarn.

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COPPER DEFICIENCY OF ONIONS GROWN ON PEAT. I. PRELIMINARY REPORT

By A. F. R. ADAMS, Canterbury Agricultural College, Lincoln, New Zealand

(Received for publication, 23rd June, 1948)

Summary

A preliminary account is given of a copper deficiency of vegetables, particularly onions, grown on an acid peat soil near Christchurch. A significant response to a foliar spray treatment using copper sulphate has been obtained. A more extensive trial is proceeding.

INTRODUCTION

Historical

THE stimulating effect of copper on plant growth, particularly on peat soils, has been noted by many workers. Felix (1) obtained improved growth of plants by the application of copper sulphate, both as a soil amendment and as a foliar spray, and Allison, Bryan and Hunter (2) were able to produce crops on previously unproductive peat by the use of copper sulphate. Similarly Bryan (3) obtained greening in chlorotic leaves of plants from the Florida Everglades soil by treatment with solutions of copper and manganese salts. In Northern Europe, Ritzema (4) and Hudig, Meyer and Goodyk (5) showed that copper sulphate was effective in controlling " Reclamation disease " which occurred on soils largely rich in humus.

However, these experiments and many others, while indicating the beneficial effect of copper on the plant, did not establish final proof of its essentiality to plant growth. The first evidence in this direction was given by Sommer (6). Using specially purified salts she showed that tomato, sunflower and flax plants failed to make normal growth in the absence of copper. Under similar conditions Lipman and Mackinney (7) showed that barley was unable to produce seed. Since that time, solution culture work has shown the necessity of copper for the normal growth of a wide range of plants.

It is now generally accepted that copper should be placed in the category of essential elements.

The use of copper salts applied as sprays to the crop or as an application to the soil to correct deficiencies is now widely used in many areas in other countries, especially on market garden crops grown on peat soils.

To date there is no record in New Zealand of deficiencies of copper affecting plant growth, though Cunningham (8) has demonstrated the effectiveness of applications of copper sulphate in correcting peat scours in cattle and enzootic ataxia in sheep grazed on copper deficient pastures on peat soils in the Waikato and other areas of New Zealand.

EXPERIMENTAL

Although vegetables for the Christchurch market are grown in many areas, the production from the peats and peat loams round the Marsh-land district represents a major portion of the total. Much of the most intensively farmed peat areas has been cropped for upwards of forty years but additional blocks have been cleared and developed within the last ten years.

On a visit in January, 1947, to a farm on one of the recently developed blocks of acid peat soil (pH 4.5—5.0, with 70 per cent. organic matter) it was noted that the crop of onions was very poor and showed many patches where the plants had made practically no growth and others where they had declined after approximately eight weeks' growth (4-6 inches).

The symptoms suggested the probability of trace nutrient deficiency. A trial area was marked off and replicated plots were given spray applications of the following elements: manganese, zinc, copper, molybdenum, boron, magnesium, nitrogen, phosphorus and potassium. At the time of application all the onions were in very poor condition, the majority showing the symptoms described later. The trial area was visited one month later and the following results noted.

With the exception of the copper treated plots, no visible improvement was apparent; in fact, in most instances the plants had deteriorated. The two copper treated plots showed a marked improvement.* Though at the time of treatment the plants were dying back and in many instances were very nearly dead, they had made a remarkable recovery and considerable new growth was evident (Fig. 1).

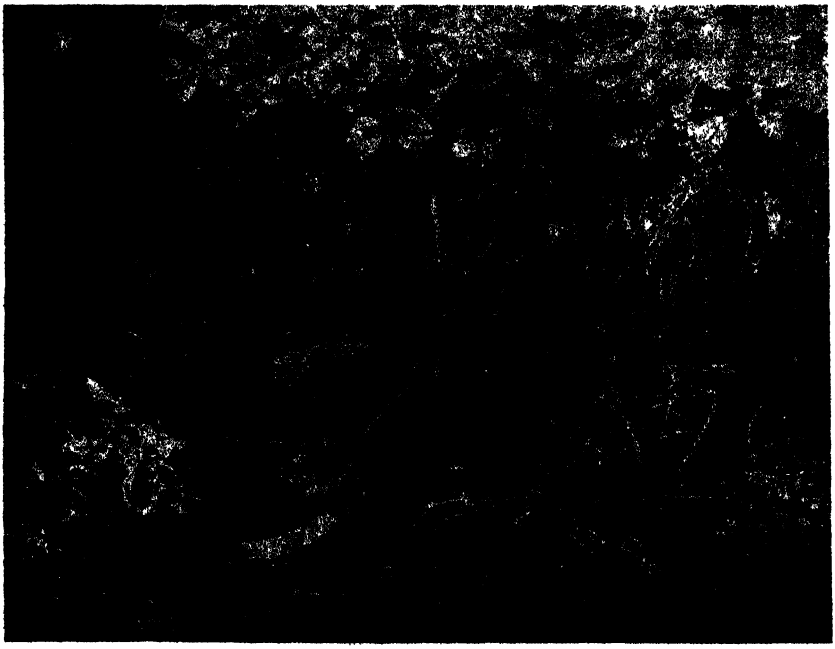

FIG. 1.—Copper deficiency of onions.
Left, portion of control plot Right, portion of copper treated plot.

The mode of application of the copper was as follows:— One ounce of copper sulphate was dissolved in 2½ gallons of water, a wetting agent added and the solution watered directly on to the plants. This quantity was used on an area of 15 sq. yds.

Fig. 2 shows typical onions from the copper treated and control plots one month after treatment. The marked response is clearly shown, both in foliage and in the development of the bulb and roots.

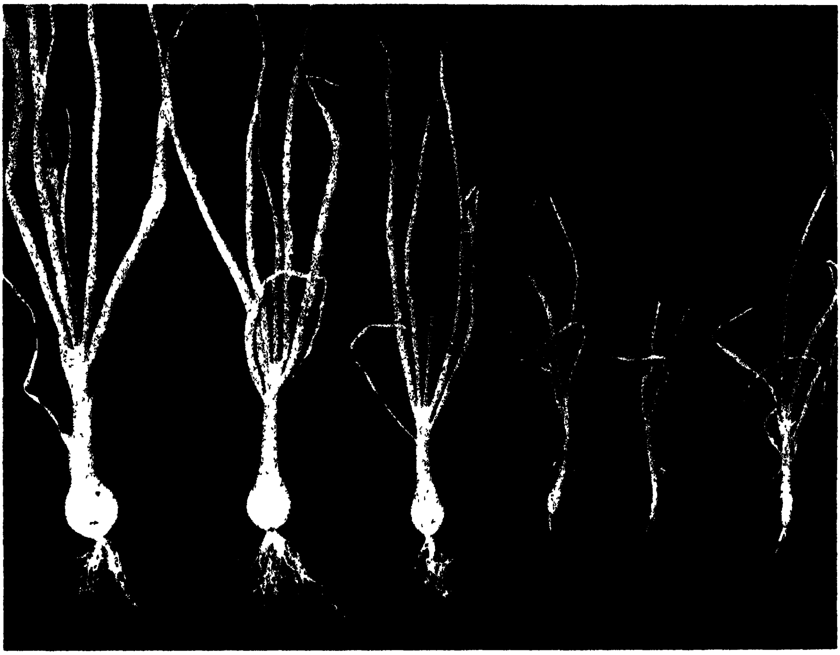

FIG. 2 Copper deficiency of onions
Left, copper treated onions one month after treatment
Right, control No copper

SYMPTOMS OF COPPER DEFICIENCY

The physiological symptoms of copper deficiency for some plants, such as cereals and citrus trees, have been fully described in the literature, but those for onions have received less attention from workers in the field. However, Harmer (9) quotes poorly coloured onions and tops dying back prematurely from the tips as indicative of copper deficiency and these symptoms are confirmed by Knott (10) and Muckenhirn (11). J. J. Skinner in Hambidge (12) lists the following symptoms as characteristic : leaves failing to maintain firmness, and foliage chlorotic with a bleached appearance ; while Brenchley (13) states that chlorosis of leaf tips is very usual, and cites poor growth, with many plants failing to reach maturity as other symptoms which sometimes occur in cases of copper deficiency of plants. The symptoms observed during the course of the trial agreed with those quoted above and can be summarized and extended as follows :

Leaves.

Somewhat paler than usual with some slight chlorotic mottling, accompanied by loss of turgor (the older leaves being most affected), from the tips downwards. These leaves later bend back, wither and die back to the point of bending.

Bulb and root development.

Bulbs poorly developed, the majority failing to mature, with many actually going back in development and finally withering. Root system very weak.

COPPER CONTENT

The above evidence for a deficiency of copper is further supported by analyses of the onions for copper content, using the method of Clare, Cunningham and Perrin (14). The copper contents of three samples of onions from the trial area and of one sample from the treated plots are given in Table I. Included also in this Table are figures for a normal onion crop grown on a different soil type and figures cited by other workers.

TABLE I. COPPER CONTENT OF ONIONS
(p.p.m. on moisture-free basis)

Untreated Onions.	Treated Onions.	Normal Onions.	Lindow, Elvehjem and Peterson (15)	Remington and Shiver (16).	Coleman and Ruprecht (17)
4.5* 2.8 3.2	7.1*	9.2† 8.6	13.5†	11.5†	13 16†

* Whole plants. † Bulb only.

Other crops, viz., lettuce and red beet, growing on similar soil, showed poor growth and it was thought probable that this was another manifestation of the same trouble. Carrots, swedes and potatoes appear to be less affected than are other vegetable crops.

A more extensive trial to measure the relative effectiveness of foliar spray and light and heavy soil dressings was laid down on this area in September, 1947, and work on this trial is proceeding. Marked yield responses to applications of copper sulphate have been obtained and it is proposed to deal fully with this work in a paper to be published later.

ACKNOWLEDGMENTS

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SPECTROPHOTOMETRIC DETERMINATION OF COBALT IN PASTURES AND ANIMAL TISSUES

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(*Received for publication 23rd October, 1947*)

Summary

(1) A procedure for the preparation of solutions for spectrophotometric measurement of cobalt is described Ether extraction of iron is necessary

(2) Absorption spectra of the cobalt complex and the reagent, before and after nitric acid treatment are presented

(3) The data suggest that for direct application of spectrophotometric methods, measurements are most satisfactory at 540mμ provided the solutions are shielded from daylight

(4) For solutions freed from excess reagent by treatment with bromine-water measurements are best made at 480mμ Special precautions to exclude daylight are unnecessary Bromine-water treatment produces no marked change in the absorption spectrum of the cobalt complex

Introduction

The main difficulties encountered in the visual measurement of the colour of the cobalt complex with nitroso R-salt (1) (2) result from the presence of the residual colour due to excess reagent. The partial destruction of the excess reagent by nitric acid introduces a very labile yellowish component, the intensity of which is affected by the amount of nitric acid used, the final boiling time, the volume of the final solution, the amount of buffer added or of salts present, the age of the solutions and the degree of exposure to sunlight.

Possible methods for eliminating these difficulties due to excess reagent are: (a) reduction in amount of reagent used, (b) elimination of the final nitric acid treatment, (c) complete destruction of the excess reagent colour, (d) use of spectrophotometric methods. The first three methods have been found to be unsatisfactory for the following reasons:—

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(a) It is not safe to reduce the amount of reagent used since (i) the interaction between cobalt and nitroso R salt depends on an endothermic time reaction reaching equilibrium under the particular conditions employed hence an excess of reagent is desirable to ensure equilibrium at very low cobalt levels, (ii) the test solutions contain ions which from various evidence (1) (3) appear to form comparatively stable complexes at the reactive pH's for the formation of the cobalt complex and therefore immobilize part of the reagent, (iii) reducing and oxidizing agents readily attack the reagent. Even the 1 ml 5 per cent nitric acid added to the solutions just prior to the colour development (2) oxidizes part of the reagent as shown by its effect on the equilibrium colour intensity produced with a fixed amount of reagent by excess cobalt.

(b) It is not convenient to dispense with the final acid treatment as usually a solid phase separates from the test solutions at the reactive pH range and the excess nitric acid is required to give clear solutions. Possibly some other strong but non-oxidizing acid such as hydrochloric acid may be suitable for this purpose but the solutions will still contain an interfering yellowish colour due to unaltered excess reagent and will be unsuitable for visual matching without filters.

(c) Marston and Dewey's method (3) of destroying the excess reagent with bromine water was applied but traces of iron remaining in test solutions even after ether extraction were found to interfere at low cobalt levels by changing the colour from faint pink to orange thus making visual matching impossible. Citrate or pyrophosphate failed to suppress the iron colour even when added to the limits of solubility probably because the solutions were so strongly acid. However under good light conditions the eye can usually match the colours readily and with satisfactory accuracy when the excess reagent colour is present. This is because the background colour due to the excess reagent is sufficiently intense to be unaffected by the presence of variable small amounts of iron as found in the normal method.

(d) As it is desirable to eliminate subjective methods in the measurements particular attention has been devoted to spectrophotometric methods with the results reported in this paper.

EXPERIMENTAL

Absorption spectra of pure solutions

Cobalt nitroso R Complex

Absorption curves obtained by means of the Hilger medium quartz spectrograph are shown in Fig. 1. The cobalt complex in solutions containing no added nitric acid, shows a peak at about 410mμ (ϵ about 48 000) a minimum at 348mμ (ϵ = 28 000) a minor peak at 318mμ (ϵ = 52,000) a minor minimum at about 302mμ (ϵ = 48 000) and beyond this the absorption increases rapidly to extremely high values in the far ultraviolet. When nitric acid is present in the amounts used in the normal method (1) a similar curve is obtained to 340mμ beyond which observations cannot be made owing to strong absorption by the nitric acid. The molecular extinction coefficient, ϵ, of the cobalt complex, deduced from absorption curves obtained with excess cobalt, excess reagent and reagent alone is approximately 43,000 at the 410mμ peak. Observations at wave lengths longer than 440mμ were made on the Cenco-Sheard spectrophotelometer with 7mμ wave band.

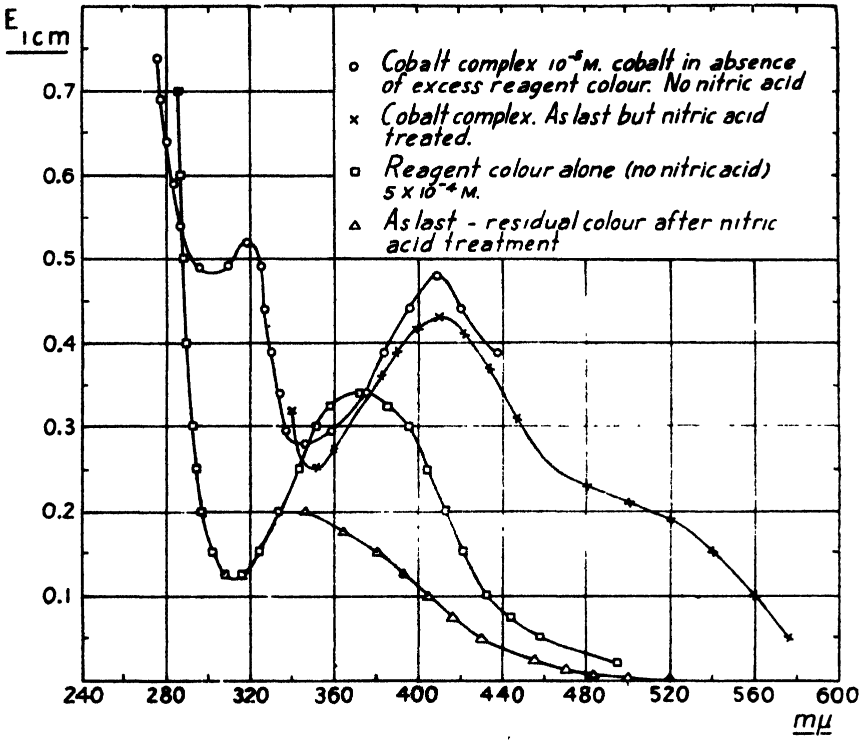

FIG. 1

Exact values for the molecular extinction coefficient of the cobalt complex have not been obtained partly because of difficulties experienced in the preparation of a reagent free from isomers and with a known amount of bound water. Although the kinetics of the reaction are imperfectly understood, it appears that the development of the cobalt complex depends on an equilibrium reaction, the complex being " fixed " by boiling with nitric acid. Different values for the apparent molecular extinction coefficient have been obtained at equilibrium according to the amount of excess reagent when the cobalt was kept constant or the amount of excess cobalt when the reagent was kept constant. When the reactant which was required in smallest amount, namely cobalt, was added in very great excess the equilibrium was shifted furthest towards a postulated 100 per cent. colour development. The complexity of the reaction may be still further accentuated if the suggestion (5) receives confirmation that part of the reagent, on the analogy of α -nitroso β -naphthol, is used up to oxidize the cobalt from the divalent to the trivalent form. However, this apparent need for quantities of reagent larger than the theoretical amount may merely be an equilibrium effect. Peroxide treatment of an acetic acid solution of cobalt, as used by Mayr and Feigl (6) for α -nitroso β -naphthol, failed to reduce the amount of reagent necessary for maximum colour development with a fixed amount of cobalt. In the tests carried out, more was needed, presumably because of partial destruction of the reagent by oxidation.

Reagent.

For this work the Hilger medium quartz spectrograph, the Cenco-

Sheard spectrophotelometer (7 to 15mμ wave bands) and the Coleman spectrophotometer (35mμ wave band) have been used, but the results shown in the figures were obtained with the first two instruments. The curves extending into the ultraviolet beyond 340mμ were obtained by the Hilger instrument. The wide band of 35mμ on the Coleman spectrophotometer precludes exact measurement of the extinction coefficient.

The absorption curve for the reagent, in the absence of nitric acid, shows a broad peak at 372mμ (ϵ = 6,800), a minimum at 312mμ (ϵ = 2,400) and beyond this sharply increases to high values in the far ultraviolet. After nitric acid treatment, the reagent peak at 372mμ is eliminated and the absorption steadily increases from 540mμ to the limit of observations at 340mμ. In such solutions daylight shifts the absorption by excess reagent strongly towards the longer wavelengths but beyond 540mμ this colour change is negligible (Fig. 2). This shows the calculated absorption curves for the amount of reagent used in the method and for a typical amount of cobalt, as found in pastures and animal tissues. The graph clearly shows the light labile nature of the excess reagent colour.

Cobalt complex in presence of excess reagent.

Reference to Fig. 2 shows that, under the conditions specified in the method (2), the excess reagent colour absorbs so strongly at the 410mμ peak as to render measurement at this wavelength impracticable with amounts of cobalt below about 10μg. At 560mμ the excess reagent colour shows very slight absorption, whereas the cobalt colour still absorbs appreciably, the extinction being about 23 per cent. of that at the 410mμ maximum. The effect of light on the excess reagent is particularly marked at the shorter wave lengths from about 520mμ and the absorp-

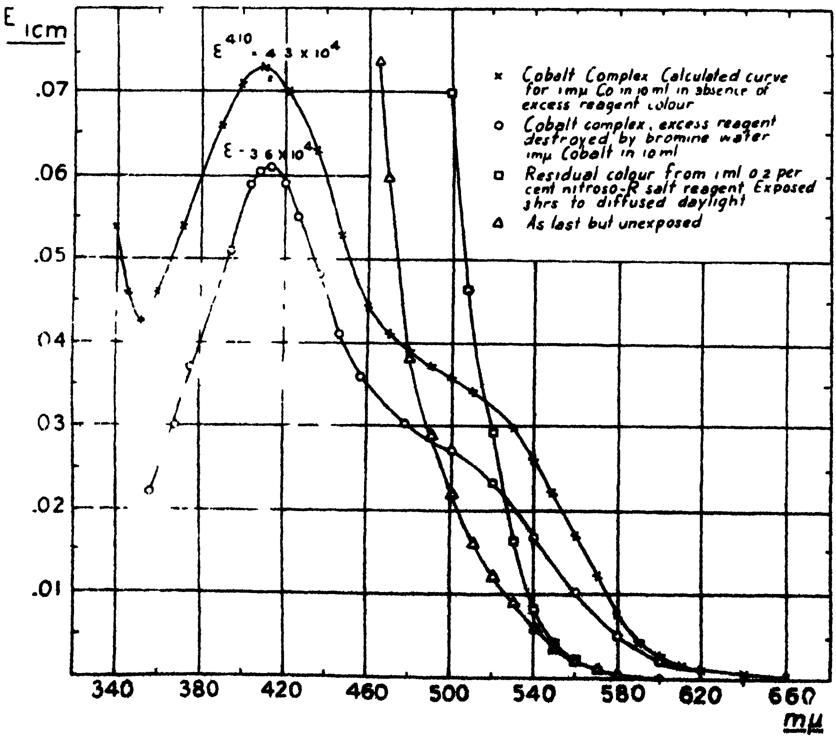

FIG. 2

tion curve rises sharply. Consequently the effective range over which estimation can safely be carried out with the spectrophotometer in this method is limited to about 520 to 580mμ. Only at wavelengths longer than 560mμ is absorption by the reagent unaffected by light. As the investigations into cobalt deficiency demand measurements at least down to 0.2μg. cobalt in 10 ml., 540mμ appears to be the most practicable wave length to use in the method outlined. The early model Cenco-Sheard spectrophotelometer with 1 cm. cells and the Coleman " Universal " photoelectric spectrophotometer were not quite as sensitive as desired, when used in the normal way. With the former instrument set at 540mμ and 10 to 15mμ wave band, scale readings had to be used between 90 and 100, outside the accurate range of the instrument. With the latter, measurements could be made at 540mμ (35mμ wave band) down towards 0.2μg. cobalt, provided the null deflection method was used. Greater accuracy should be possible with cells of longer light path or even by boosting the final colour intensity by addition of a known amount of cobalt, e.g. 1μg. to the test solutions just prior to the colour development. In all the above work shielding the solutions from daylight is necessary, more particularly if measurements are made at wave lengths shorter that 550mμ.

Solutions treated with bromine-water.

The similarity in absorption curves to those for untreated solutions containing excess cobalt (see Fig. 2) indicates that the bromine-water treatment produces no radical change in absorption spectrum of the cobalt complex over the range from 660 to 340mμ, though the molecular extinction coefficient appears to be reduced from about 43,000 to about 36,000 at the 410mμ peak.

Absorption spectra of pasture and liver ash solutions

Especially in the ultraviolet, the absorption spectra of pasture and liver ash test solutions were found to differ slightly from those of the standards, owing to traces of iron remaining even in ether extracted solutions.

The difference in absorption of light by ferric iron and by the cobalt complex was found to be sufficiently great, however, to enable estimations to be carried out between 460 and 560mμ, provided the bulk of the iron was removed by preliminary ether extraction. On the Coleman instrument results obtained with a typical amount of iron normally present in 10 g. " clean " pasture, namely 1mg., were about 13 per cent. high at the 1μg. cobalt level using 480mμ (35mμ band), and about 65 per cent. high at the 0.2μg. level. After ether extraction, the amount of iron remaining did not exceed 0.2 mg. At this level the interference was less than 3 per cent. for 1μg. cobalt and 13 per cent. for 0.2μg. cobalt, both these errors being within the apparent limits of accuracy of measurements at these levels. From the above considerations it appears that ether extraction of iron offers distinct advantages in the preparation of the test solutions, whether for direct spectrophotometric measurement or for measurement after destruction of excess reagent. In the latter method the wave-length of 480mμ appears to be the most satisfactory as the absorption curve of the cobalt complex is fairly flat and the absorption reasonably high.

Reference to Fig. 1 shows that, if some reliable treatment other than that with nitric acid, can be devised to give clear test solutions, it may be possible to carry out direct measurements with instruments such as the Beckmann spectrophotometer at 312mμ. At this wave-length, in solutions containing no nitric acid, absorption by the excess reagent is at a minimum and the curve for the cobalt complex is comparatively flat.

Conclusions

The absorption spectra of the test solutions after ether extraction of iron do not differ significantly from those of pure standard solutions at the selected wave-lengths.

For direct application to the determination of the cobalt complex in the presence of the excess reagent colour, measurements are best made at about 540mμ, shielding the solutions from daylight. With longer wave-lengths interference by the excess reagent colour is much reduced, but any advantage from this is offset by the diminished absorption by the cobalt complex. For higher levels of cobalt than are normally found in pastures and animal tissues, shielding from daylight is unnecessary if readings are made between 560 and 600mμ, as the excess reagent colour is not affected by daylight over this wave band.

Measurement of the colour intensity in solutions freed from excess reagent by means of bromine-water is best made between 460 and 500mμ. As the excess reagent is absent, no special precautions to shield the solutions from daylight are needed but exposure to direct sunlight should be avoided. Shorter wave-lengths than 460mμ cannot safely be used, owing to increasing absorption by the traces of iron remaining in the solutions even after careful ether extraction. Even over the recommended range iron detectably interferes when its ratio to cobalt is greater than 100 : 1.

METHOD

On the basis of the above experimental data and conclusions, the following method has been developed.

Ashing:

Kidson and Askew's ashing method (4) is used.

Preparation of solutions:

For pastures a modified procedure is necessary to eliminate iron. The ash is taken up in 20 ml. constant boiling point (20 per cent.) hydrochloric acid, and the solution plus residues transferred to a beaker. The contents are evaporated fully to dryness on the sandbath and again taken up by boiling with 20 ml. 20 per cent. hydrochloric acid and filtered, washing thoroughly with hot water. Three drops of concentrated nitric acid are added and the solution is concentrated till pungent fumes of hydrochloric acid are evolved. The cooled solution is transferred to a separating funnel, washing in with 20 per cent. hydrochloric acid. Iron is separated by two extractions with ether. The solution is evaporated to small volume, 1 ml. concentrated nitric acid added to destroy organic matter introduced with the ether and the contents evaporated fully to dryness on the sand bath to eliminate the aqua regia. (*Note:* Solutions at the stage just prior to the colour development must be kept away from these fumes). A permanganate-like colour due to manganese usually develops under these conditions. Repeated evaporation to dryness with hydrochloric acid may be required to eliminate this compound, which, if not destroyed, may subsequently interfere by oxidizing part of the reagent. To the cooled residue are added 10 to 15 ml. of water, followed by 0.5 ml. of 20 per cent. hydrochloric acid, etc., as in the normal method (2). For animal tissues no change in procedure is necessary.

Colour development is carried out by the method previously described (2).

Colour measurement :

Two procedures are available

(a) Direct spectrophotometric measurement · In the presence of the excess reagent colour measurements are made at 540mμ, the solutions shielded from daylight.

(b) Spectrophotometric measurement after destruction of excess reagent : The excess reagent is destroyed by the method of Marston and Dewey (3) ; 1ml. 0.1N bromine-water is added to the hot solution after boiling with nitric acid. The solution is allowed to stand for 5 minutes and then boiled for 1 minute to eliminate excess bromine Measurement of the colour intensity is made at 480mμ.

ACKNOWLEDGMENTS

Thanks are due to Mr. W. S. Metcalf, lecturer in Physical Chemistry, Victoria University College, Wellington, for advice and assistance on the spectrographic and spectrophotometric methods. The co-operation of the Fields Division Chemical Laboratory, Soil Fertility Research Station, Hamilton, in making available the Coleman spectrophotometer is also gratefully acknowledged.

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SOIL DISINFECTION

VII. COMPARATIVE VALUE OF FORMALDEHYDE AND OF PARAFORMALDEHYDE IN CONTROL OF VERTICILLIUM-WILT

By H. JACKS, Plant Pathologist, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 9th May, 1947*)

Summary

Formaldehyde and paraformaldehyde were tested for control of verticillium-wilt of tomatoes.

Formaldehyde at a concentration of 0.8 per cent. (equivalent to 2 per cent. formalin solution), gave effective control of verticillium-wilt.

Paraformaldehyde was not as effective at equivalent dosages but gave satisfactory results at higher concentrations.

Paraformaldehyde caused less plant damage than did formaldehyde at equivalent dosages. Damage with either material was negligible, provided planting was delayed until 3 to 4 weeks after application.

INTRODUCTION

REPORTS received from growers suggested that the recommended formaldehyde treatment of 2 per cent. formalin (40 per cent. formaldehyde), applied at rate of 50 gallons to 15 sq. yd. of soil did not give effective control of verticillium-wilt (*Verticillium albo-atrum R and B*), in tomato crops. It was also reported that formation of paraformaldehyde precipitate which sometimes occurs during storage, resulted in a loss of efficiency and caused damage to plant growth. Investigations described in this paper were undertaken to ascertain the comparative toxicity of formaldehyde and paraformaldehyde to verticillium-wilt of tomatoes and to determine their effect on plant growth.

METHOD OF INOCULATION OF SOIL

Isolations from diseased plants were cultured on prune agar, transferred on to potato dextrose agar and finally grown on a media designed for easy mixing with soil. The latter media was prepared by mixing one part of oatmeal with 4 parts of sand. This mixture was placed in deep petri dishes at 3 oz. per dish, moistened and autoclaved for 2 hours at 15 lb. pressure. Dishes were left to cool, inoculated with culture and kept in an incubator at 25°c. until such time as the fungus had grown throughout the media. This was completed 25 days after inoculation. Steam sterilized potting soil was infected by mixing 6 dishes of inoculum with each cubic foot of soil. This was left for three weeks to allow the fungus to spread throughout the soil volume and, before treatment, was placed into boxes each of ½ cu. ft. capacity.

MATERIAL AND METHOD

Paraformaldehyde supplied in compressed tablets was ground into a fine powder and dissolved in a known volume of warm water. Formalin (40 per cent. formaldehyde) and paraformaldehyde solutions were watered on to the soil at the rate of ½ gallon per box and in proportions listed in Table I. The same quantity of water only, was applied to checks. Treatments were replicated four times.

COMPARATIVE EFFECT OF FORMALDEHYDE AND PARAFORMALDEHYDE

Soil in boxes was treated on 18th September, 1945, planted on 9th October with 24 Break O'Day tomato plants per box and results were recorded on 16th December.

Infected plants showed stunted growth, discoloration of vascular tissues and yellowing followed by wilting of lower leaves. Results recorded as percentage infection and weight of aerial portion of plants are given in Table I.

TABLE I. EFFICACY OF DIFFERENT CONCENTRATIONS OF FORMALDEHYDE AND PARAFORMALDEHYDE IN CONTROL OF VERTICILLIUM-WILT OF TOMATOES

Treatments carried out on soil held in boxes. Results from four replications of 24 plants each set out 3 weeks after treatment.

Percentage Concentration	FORMALDEHYDE		PARAFORMALDEHYDE.	
	Av Per cent Infection *	Mean Weight of Plants (grams)	Av Per cent Infection *	Mean Weight of Plants (grams)
0.0	91.66	9.52	91.66	9.52
0.1	59.74	13.62	65.70	12.17
0.2	41.40	13.20	61.46	9.89
0.4	39.37	11.93	51.04	10.97
0.5	12.46	11.04	27.70	11.56
0.8	3.12	15.05	25.00	13.84
1.6	0.00	6.62	5.49	12.71
3.2	0.00	6.12	0.00	7.56
Standard Error.	5.06	2.40	6.55	0.85
Difference Required for Significance at 5 per cent. level.	14.73	7.00	19.09	2.60

* Re-isolations made from vascular tissues of infected plants produced cultures of *Verticillium albo-atrum* in every case

All treatments gave significant reduction of the disease when compared with untreated soil, formaldehyde at 0.8 per cent. and over and paraformaldehyde at 1.6 per cent. and over giving satisfactory control. Although differences in plant weight did not reach significance at 5 per cent. level, results suggest that formaldehyde at 1.6 and 3.2 per cent. and paraformaldehyde at 3.2 per cent. had a detrimental effect on plant growth.

TABLE II. MORTALITY OF TOMATO SEEDLINGS IN SOIL TREATED WITH DIFFERENT CONCENTRATIONS OF FORMALDEHYDE AND PARAFORMALDEHYDE AND PLANTED 10, 15, 20 AND 25 DAYS AFTER TREATMENT RECORDS FROM 3 REPLICATIONS OF 36 PLANTS EACH

Percentage Mortality in Plants.

Percentage Concentration.	FORMALDEHYDE.				PARAFORMALDEHYDE.			
	Period Between Treatment and Planting.				Period Between Treatment and Planting.			
	10 Days.	15 Days	20 Days	25 Days.	10 Days	15 Days	20 Days.	25 Days
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	9.37	4.17	1.04	0.00	2.08	1.04	0.00	0.00
0.8	41.67	7.28	1.38	0.00	4.16	2.18	0.00	0.00
1.6	100.00	55.21	27.08	2.08	7.29	3.25	0.00	0.00
3.2	100.00	84.37	30.21	3.12	98.96	31.25	8.33	5.21

EFFECTS OF FORMALDEHYDE AND PARAFORMALDEHYDE ON GERMINATION AND PLANT GROWTH

Boxes containing steam disinfected soil were treated with solutions of formaldehyde or paraformaldehyde at concentrations of 0.4, 0.5, 0.8 and 1.6 per cent. The soil was in a suitable condition for sowing 14 days after treatment and 500 tomato seeds were then sown in each box. All treatments were replicated three times. The number of seedlings that emerged was recorded after a period of 16 days. Viability of tomato seed was not affected by the treatments and resultant seedling growth was more vigorous than in untreated soil. Germination of seed sown in the higher concentrations (0.8 and 1.6 per cent.) of both materials was delayed by 2 to 3 days.

Boxes treated with the various concentrations of formaldehyde and paraformaldehyde were planted with 36 Potentate tomato seedlings at 10, 15, 20 and 25 days after application of solutions. Records of plants injured by residual effects of treatments were taken after 5 days (see Table II).

Injury to plants occurred in soil treated with concentrations higher than 0.4 per cent., but paraformaldehyde permitted earlier planting of soil than formaldehyde. In all cases soil was reasonably safe for planting 3 to 4 weeks after treatment.

DISCUSSION

Concentrations of formaldehyde at 0.8 per cent. and over gave satisfactory control of the fungus. Paraformaldehyde at twice the strength of formaldehyde solutions gave approximately equivalent results. Soil treated with paraformaldehyde solutions could be planted one week earlier than soil treated with equal dosages of formaldehyde. Neither material damaged plants set out 3 to 4 weeks after treatment.

SOIL DISINFECTION VIII. CHEMICAL CONTROL OF VERTICILLIUM-WILT OF TOMATOES

By H. JACKS, Plant Pathologist, Plant Diseases Division, Department of Scientific and Industrial Research

(*Received for publication, 17th July, 1947*)

Summary

Experiments on control of verticillium-wilt of tomatoes by soil treatments showed that:—

(1) Chloropicrin and formalin gave satisfactory control both in potting soil and in glasshouses.

(2) Carbon disulphide, D-D, Iscobromes I and II were of value in reducing incidence of the disease.

(3) Chloropicrin, D-D, carbon disulphide and Iscobromes I and II applied as emulsions to soil in boxes, gave better control than application of concentrated fumigants to soil held in gastight containers.

(4) Some fumigants gave better control when applied as mixtures.

INTRODUCTION

INVESTIGATIONS were carried out to ascertain the efficacy of soil fumigants in controlling verticillium-wilt of tomatoes (*Verticillium dahliae* Kleb).

Treatments were applied either to soils artifically infected or to those which had previously carried diseased crops.

Trials described in this paper cover investigations on control of verticillium-wilt by soil fumigants. Effects on plant growth and yield of tomatoes are also recorded.

MATERIALS AND METHODS

Composition of materials used and method of preparation of emulsions have been given in earlier papers (Jacks, 1946; 1947). Methods of applying materials were similar to those previously reported (Jacks, 1945 A; 1945 B). Dosage rates are given in the tables. Preparation of artificially inoculated soil has been described previously (Jacks, 1947).

The relative merits of fumigants were determined by (a) the presence or absence of fungus in vascular tissues, (b) the top weight of tomato seedlings, and (c) fruit yield of first five trusses.

Effects of chloropicrin on control of verticillium-wilt and yield of tomatoes

A. Applied to potting soil

Infected potting soil, sufficient for four boxes (dimensions 12 in. x 18 in. x 6 in.), was treated with chloropicrin at 5 ml. per cu. ft. and held for 48 hours in closed containers. The soil was then transferred to the boxes and 14 days after treatment each of these was planted with 24 Kondine tomato plants.

Results.—Records taken 14 weeks after planting showed 87.25 per cent. infected plants in untreated soil and 100 per cent. healthy plants in chloropicrin treatment. Plants in the checks became stunted and wilted at the tops, followed later by yellowing and wilting of bottom leaves. Those in treated soil remained healthy and produced good growth.

B. Applied to soil in situ

Chloropicrin was applied to soil in a commercial glasshouse in which the previous crop had shown approximately 25 per cent. infected plants. The material was injected at the rate of 2 ml. per sq. ft. to a depth of 5 in. Seven days after treatment 72 Potentate tomatoes were set out in each of six treated and six untreated plots.

Results.—The short period allowed for aeration of the soil caused a slight check in the growth of plants from residual chloropicrin, but recovery was complete after two weeks. Differences in growth between plants in treated and untreated soil were not apparent for the first two months. Later it became necessary to replace some wilted plants in untreated plots. Final records showed 48.5 per cent. infection in untreated plots, with an average yield of 4.9 lb. of fruit per plant, while records of treated plots showed 6.2 per cent. infection and 7 lb. of fruit per plant. In this experiment chloropicrin failed to give the complete control of verticillium-wilt obtained in a previous trial (Jacks, 1945), but nevertheless gave an increase in yield and considerably reduced incidence of the disease.

Effects of chloropicrin, D-D and formalin on control of verticillium-wilt and yield of tomatoes

Light volcanic loam in a glasshouse was artificially infected with cultures of *Verticillium dahliae*. The area was divided into 16 plots of 110 sq. ft. each, which were separated by asbestos boards sunk to a depth of 14 in. Formalin solutions were applied to the soil surface with a watering can, while fumigants were injected 5 in. deep. Treatments were applied on 22nd June, 1945, plots being randomized and replicated four times. Thirty-six Potentate tomato plants were set out in each plot on 12th July and harvesting records were completed by 4th February, 1946.

In the following season (1946), the experiment was repeated in the same house. The soil was again infected artificially and treatments were applied six weeks later. Crop yields were not recorded but degree of infection was determined by examination of vascular tissues in November, 1946. Results of trials are given in Table I.

TABLE I EFFECT OF CHLOROPICRIN, D-D AND FORMALIN IN CONTROLLING VERTICILLIUM-WILT OF TOMATOES AND ON THE WEIGHT OF FRUIT PER PLANT

Treatment	Quantities per sq ft (ml)	Season 1945		Season 1946
		Mean percentage infected plants	Mean weight of fruit per plant (Pounds)	Mean percentage infected plants
Check	0.0	45.55	5.68	62.49
Chloropicrin	2.0	7.63	8.19	6.10
D-D	2.0	18.72	6.99	23.79
Formalin	33.6	15.80	6.01	8.51
Difference required for Significance at 5 per cent level		11.43	1.84	11.25
Standard error		3.57	0.57	3.52

Results.—In 1945, height of tomatoes was even throughout the glasshouse for the first two months, after which chloropicrin treatment showed slightly better growth. All treatments gave an increase in yield over checks but only in the case of chloropicrin was this increase statistically significant. In both years, treatments gave significant reduction of infection, with chloropicrin giving most effective control. In both years, dry conditions at time of application affected the dispersion of formalin solutions, thus reducing efficacy of the fumigant in controllling the disease.

Effects of chloropicrin, D-D, carbon disulphide and iscobromes I and II on control of verticillium-wilt and growth of tomato seedlings

Two experiments were carried out (a) to determine comparative effects of concentrates and (b) to determine effects of the same fumigants applied in emulsified form.

Expt. (a). Application of concentrates

Infected potting soil, in sufficient quantity to provide four replications per treatment, was treated in air-tight containers and transferred to boxes after 48 hours. Ten days after treatment 24 Kondine tomatoes were planted in each box. Records of infection and weight of aerial parts of plants were taken after ten weeks and are given in Table II.

Results.—Plant injury appeared within 24 hours of planting, percentage mortality of seedlings being given in Table II. Further injury occurred in the next four days, i.e. 15 days after treatment, after which the fumigants had no further effect on seedling mortality.

TABLE II. EFFECTS OF SOIL FUMIGATION WITH CHLOROPICRIN, D-D, CARBON DISULPHIDE AND ISCOBROMES I AND II ON CONTROL OF VERTICILLIUM-WILT, WEIGHT OF TOMATO SEEDLINGS AND PLANT GROWTH

Treatment	Quantities per cu. ft. (ml)	Mean Percentage infected plants.	Mean weight of aerial parts of plants (g)	Percentage mortality after treatment 11 days	Percentage mortality after treatment 15 days
Check	0.0	96.87	10.33	0.0	0.0
Chlotopicrin	6.0	0.00	19.44	1.1.	0.3
D-D	6.0	66.66	12.53	4.3	1.8
Carbon disulphide	15.0	56.24	11.23	0.3	0.0
Iscobrome I*	6.0	68.74	13.03	4.1	2.4
Iscobrome II**	6.0	51.04	12.46	2.6	2.4
Difference required for significance at 5 per cent. level		17.80	3.08		
Standard error		6.28	1.04		

* Iscobrome I: 15 per cent methyl bromide and 85 per cent. xylol

** Iscobrome II: 25 per cent. chloropicrin, 15 per cent methyl bromide and 60 per cent. xylol

Chloropicrin gave significant improvements in disease control and increased weight of plants when compared with other treatments. Carbon disulphide, D-D and iscobromes I and II gave signficant reducton of infection as compared with checks. Although these materials increased plant weight, only iscobrome I gave results closely approaching significance.

Expt. (b). Application in emulsified form

Emulsions prepared with Wetsit in water were applied to infected potting soil held in boxes (dimensions 12 in. x 18 in. x 4 in.), at rates shown in Table III. Ten days later 24 Kondine tomato seedlings were planted in each box. Degree of infection and aerial weight of plants were recorded after 73 days.

TABLE III. EFFECT OF CHLOROPICRIN, D-D, CARBON DISULPHIDE AND ISCOBROMES I AND II, APPLIED IN EMULSIFIED FORM, ON CONTROL OF VERTICILLIUM-WILT AND WEIGHT OF TOP GROWTH OF TOMATO SEEDLINGS

Treatments.	Quantities in ml applied with 1 gal water to each cu. ft. of soil.	Mean percentage infected plants	Mean weight of aerial parts of plants (g.).
Check	0.0	90.08	16.79
Chloropicrin	18.0	1.04	19.67
D-D	18.0	4.58	12.41
Carbon disulphide	45.0	1.04	10.29
Iscobrome I	18.0	4.21	12.86
Iscobrome II	18.0	0.00	12.35
Difference required for significance at 5 per cent. level		6.76	5.04
Standard error		2.28	1.70

Results.—All treatments gave significant reduction in infection. With the exception of chloropicrin, treatments reduced growth due possibly to the short interval between application of fumigants and date of planting.

Effects of chloropicrin, iscobrome II and mixtures of chloropicrin with D-D and of chloropicrin with iscobrome II on control of verticillium-wilt and on crop yields

Soil under glass which had previously grown an infected crop was treated with choloropicrin, iscobrome II, a 1:1 mixture of chloropicrin and D-D and a 2:1 mixture of iscobrome II and chloropicrin. Eighteen Potentate tomato plants per plot were set out in July, 1946, there being three replications of each treatment. Yield records from the first five trusses were completed in February, 1947.

TABLE IV. EFFECT OF CHLOROPICRIN, ISCOBROME II, a 1 : 1 MIXTURE OF CHLOROPICRIN AND D-D AND a 2 : 1 MIXTURE OF ISCOBROME II AND CHLOROPICRIN APPLIED TO SOIL IN GLASSHOUSE, ON CONTROL OF VERTICILLIUM-WILT AND YIELD OF FRUIT PER PLANT

Treatments	Quantities per sq. ft. (ml.).	Mean percentage infected plants.	Mean weight of fruit per plant (Pounds).
Check	0.0	67.00	2.57
Chloropicrin	2.0	20.00	5.04
Iscobrome II	3.0	15.50	3.81
Chloropicrin and D-D (1 : 1)	2.0	8.33	5.17
Chloropicrin and Iscobrome II (1 : 2)	3.0	4.17	5.08
Difference required for significance at 5 per cent. level		20.40	1.16
Standard error		6.47	0.37

Results.—Treatments, as compared with checks, gave significant reduction in infection and increase in crop yield. Crop yield from iscobrome II was signficantly less than from other materials. Mixtures gave improved control of the disease as compared with pure materials, although differences between fumigants were not significant.

DISCUSSION OF RESULTS

Of fumigants tested, chloropicrin gave the most consistent results in control of verticillium-wilt. It also improved growth and increased crop yield. Control of the disease was not always complete and results suggest that increased efficiency would be obtained by using higher dosages. Application of higher dosages, however, may not necessarily secure increases of crop yields. Plant damage occurred where a short interval was allowed between treatment and planting. Effect of residual toxicity in chloropicrin treated soil, was, however, less evident than in other materials.

Tests with formalin were not adequate. The soil was dry at the time of application and this condition affected the dispersion of the liquid.

Control of the disease was therefore less effective than would be expected under more favourable conditions of application.

D-D, carbon disulphide and iscobromes I and II gave relatively poor control of verticillium-wilt at dosages tested. Iscobrome II was the most effective of these materials. Further experiments with the different fumigants, are necessary to ascertain the possibility of obtaining economic control by application of higher dosages. Emulsified fumigants brought about results superior to those obtained by concentrates and this method of application will be investigated further.

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SOIL DISINFECTION. IX. CONTROL OF EELWORM IN OUTDOOR SOIL

By H. JACKS, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(*Received for publication, 7th August, 1947*)

Summary

In an experiment on the control of eelworm in heavily infested outdoor soil, chloropicrin and D-D applied at the rate of 3 ml. per sq. ft. gave almost complete control, while iscobrome II at the same dosage markedly reduced infestation.

Chloropicrin and iscobrome II also increased the yield of tomatoes, D-D just failing to give significant results.

Iscobrome I at the same rate of application and carbon disulphide at twice this dosage neither controlled eelworm nor increased yield.

All these fumigants significantly reduced weed population, chloropicrin giving the best control.

INTRODUCTION

EXPERIMENTS described in the present series of papers have been concerned with the application of fumigants to potting soils and glasshouse soils treated *in situ*. The need for disinfection also occurs in outdoor soils and an account of the treatment of an area of soil heavily infested with eelworm (*Heterodera marioni* (Cornu) Goodey) is given in this paper.

METHOD

For the purpose of this experiment, an area which in the previous year had carried a crop of tomatoes heavily infested with eelworm was divided into 24 plots, each 24 sq. ft. in area. To avoid spread of infestation and diffusion of fumigants, plots were separated by asbestos boards sunk to a depth of 15 inches.

Treatments included chloropicrin, D-D, carbon disulphide, iscobrome I and iscobrome II. Materials were applied on 20th October, 1946, four plots being treated with each and a similar number left untreated to serve as checks. Before treatment half a gallon of water per square yard was applied to each plot. The fumigants were then injected into the soil to a depth of 5 inches at rates given in Table I. The instrument used for application of fumigants was that described by Jacks and Wright (1946). To reduce loss of fumigants by volatilization, holes made by the injector were tamped with soil and each plot was given a water seal of one gallon per square yard. Soil temperature at the time of application was 20°c. and total soil moisture 15 per cent. Four days after treatment the soil was forked over to assist escape of residual gases. On 9th November, 1946, nine tomato plants of the variety "Potentate" were set out in each plot.

In order to assess the effect of treatments, the following plot records were taken:

(a) Percentage of eelworm-infested plants when examined at the termination of the experiment.

(b) Weight of fruit harvested from the first five trusses. This record was completed on the 15th April, 1947.

(c) Weed establishment throughout the period of the experiment, all weeds being counted as they were removed.

RESULTS AND DISCUSSION

Results are summarized in Table I.

TABLE I. EFFECT OF FIVE SOIL FUMIGANTS ON EELWORM INFESTATION, YIELD OF TOMATOES AND WEED GERMINATION

Treatment.	Quantity per sq ft. (ml.).	Percentage of infested plants	Weight of fruit per plant (lb.).	Number of weeds per sq. ft
Check	0.0	91.7	4.8	27.3
Chloropicrin	3.0	2.7	6.8	11.2
D-D	3.0	2.8	5.8	15.2
Carbon disulphide	6.0	83.3	4.8	19.8
Iscobrome I*	3.0	80.5	5.2	18.6
Iscobrome II**	3.0	45.9	6.2	16.5
Difference required for significance at 5 per cent. level		29.9	1.1	6.0
Standard Error		10.0	0.4	2.0

* Iscobrome I—15 per cent. methyl bromide and 85 per cent. xylol.

** Iscobrome II—25 per cent. chloropicrin, 15 per cent. methyl bromide and 60 per cent. xylol.

(a) *Eelworm control.* Results show that chloropicrin and D-D gave almost complete control, while iscobrome II reduced infestation by half. Plots treated with carbon disulphide and iscobrome I showed no significant improvement on check plots. Iscobromes I and II are similar mixtures, except that in the latter 25 per cent. of xylol is replaced by an equal amount of chloropicrin. It appears therefore that the partial control effected by iscobrome II was due to the presence of chloropicrin.

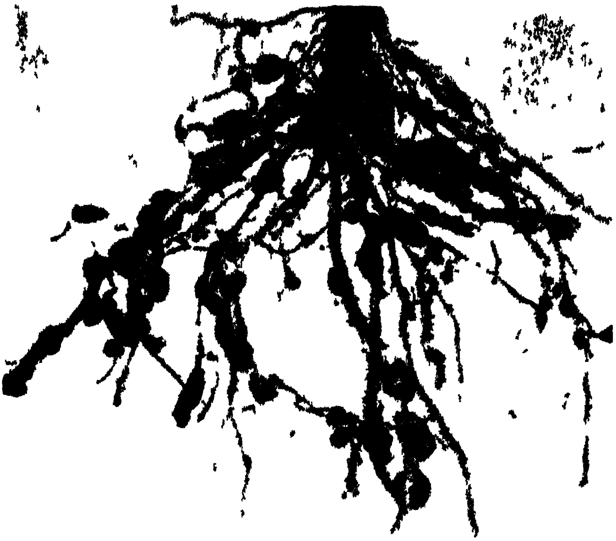

FIG. 1 (Phot. K. J. Hughes.)

Tomato root from check plots showing effect of heavy eelworm infestation × [illegible]

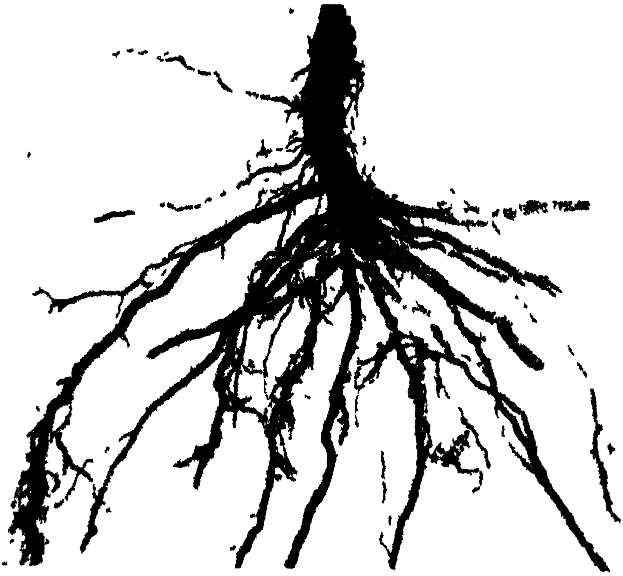

FIG. 2 (Phot. K. J. Hughes.)

Tomato root from chloropicrin plots showing effect of slight eelworm infestation × [illegible]

Additional to examination of roots for eelworm infestation, observations were made on size and number of galls. In check plots masses of galls up to ½ inch in diameter and 1 inch in length were conspicuous on most plants (Fig. 1). In plots treated with chloropicrin and D-D, galls were few and of small size (Fig. 2). In carbon disulphide and iscobrome I and II treatments, however, galls were numerous and intermediate in size between those in checks and in chloropicrin or D-D.

(b) *Yield of tomatoes.* Compared with checks, chloropicrin and iscobrome II gave significant increase in crop. D-D just failed to give a significant increase while iscobrome I and carbon disulphide were ineffective.

Although chloropicrin and D-D gave equal control of eelworm, the increase in yield was higher in the former treatment. In this connection results secured previously (Jacks, 1945) indicated that chloropicrin applied to clean soil gave a greater increase than D-D in yield of tomatoes. An improvement in the nutrient condition of the soil following use of chloropicrin was also reported by Schchepetelnikova and Cheremisova (1937) and Tam (1945). It is therefore suggested that increased production of fruit in chloropicrin treatments as compared with those of D-D may be due to improved soil fertility.

(c) *Weed control.* All treatments gave significant reduction of weed population as compared with checks, chloropicrin being the most effective. However, as only partial control was obtained it appears that reduction of weeds by soil fumigants cannot be regarded as more than an advantage incidental to their use.

ECONOMIC POSSIBILITIES

By the present injection method, approximately 1½ man hours would be required to treat 1,000 sq. ft. of area. Although effective control of eelworm under field conditions was obtained by chloropicrin and D-D, at the present time costs of labour and materials for treating other than small areas would be prohibitive. D-D being only ⅛th the price of chloropicrin, shows greater economic possibilities for control of eelworm.

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REVIEW.

BIOLOGICAL STANDARDIZATION OF THE VITAMINS.

K. H. COWARD

2nd Edition, 1947

Since its first appearance in 1937 this book has been the vade-mecum of every worker engaged in biological estimation of the vitamins. That in the second edition (1947) so little alteration to the existing text has been necessary, is in itself a tribute to the soundness and comprehensiveness of the original. Its clear statement of the principles governing biological assay, with emphasis directed to the absolute necessity, in every determination, for simultaneous trial of standard of reference and test material, remains in 1947 as in 1937, the rock on which vitamin determinations must rest.

In this edition a chapter on biological estimation of vitamin E is added to the sections on assay of vitamins A, B_1, C and D. For each of these vitamins methods of assay are fully described, preparation of animals and plan of experiment are discussed, and careful assessment is made of the merits and demerits of the various methods. For each individual vitamin also, procedure for establishment of curves of response and calculation of results on a statistical basis is explained so clearly as to be understandable even by the non-mathematically minded, while a second section of the book provides a mathematical consideration of factors influencing accuracy, and of limits of error in biological measurement.

Chemical and physical data concerning the international standard preparations are included, and in the case of vitamin A, correlation of biological with physical and chemical methods of assay discussed. Evidence of interdependence of the various vitamins is examined, and attention drawn to the need for careful planning of experiments so that statistical treatment of the results may be employed to yield information on this matter.

The book is not only indispensable to the worker on biological estimation of the vitamins, but provides stimulating reading for all those concerned with the design of animal experiments.

M. M. C.

IMPERIAL AGRICULTURAL BUREAUX

A new journal of the Imperial Forestry Bureau is now being issued entitled "FOREST PRODUCTS AND UTILIZATION." It is a reprint of *Forestry Abstracts*, Section 3.

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FURTHER INVESTIGATIONS ON THE NUTRIENT STATUS OF FLUE-CURED TOBACCO

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Summary

Harrison's Special variety of flue-cured tobacco was grown on two soil types for three seasons to determine the course of development of dry matter and of intake of nutrients by the plants

When young, the plants were well-supplied with minerals and nitrogen (on the dry-matter basis) but were relatively low in percentage of dry matter. As they developed the percentage of dry matter increased in both leaves and stalks but the concentration of minerals and nitrogen generally decreased ; lime however accumulated in leaves near the base of the plant There were distinct differences in chemical composition between leaves and stalks at all stages of growth

The maximum amount of dry matter and of minerals was found shortly after harvesting began. Production of dry matter and intake of nutrients continued during the harvesting period ; from one-third to one-half of the season's absorption of nutrients occurred at this time.

On the heavier soil intake of minerals and nitrogen was such that plants on it generally showed a higher nutrient status than those on the lighter soil.

Seasonal conditions during harvesting greatly influenced the production of dry matter and absorption of nutrients

Cured leaf from successive harvests showed appreciable differences in chemical composition ; variations from one season to another were apparently due to unidentified seasonal influences

After completion of harvesting the residual plant material is of considerable value for use as a green manure

A discussion of the present three seasons' and a previous two seasons' results is presented.

INTRODUCTION

A report on "The Nutrient Status of Flue-Cured Tobacco", published in 1944 (1), covered work carried out with plants of Harrison's Special variety in the seasons 1941-42 and 1942-43. Since that time, until the 1946-47 season, work of a similar type has been continued. Further refinements in experimental procedure have been introduced with the result that a number of points mentioned in the earlier report as requiring confirmation or elucidation can now be discussed with a greater probability of arriving at sound conclusions. Moreover the longer experimental period has enabled the effects of varying types of growing and harvesting seasonal conditions to be more clearly seen.

The present paper covers the three seasons 1944-45, 1945-46 and 1946-47. Although a similar trial was run in the 1943-44 season the results are not reported because they are not considered to be satisfactory, due mainly to difficulties that were experienced in drying the samples in that season. In the last three seasons plants of the same origin were grown on two soil types, a medium sand loam and a silt loam, to compare the development of the plants and the intake of nutrients under these two conditions.

EXPERIMENTAL

In each of the three seasons reported upon in this paper the plants for the experimental areas were provided by the Tobacco Research Station at Riwaka. Harrison's Special variety was grown. In 1944-45 season the strain was the same as that used in 1941-42 and 1942-43, but in the 1945-46 and 1946-47 seasons another strain, giving a broader type of leaf, was used. It is therefore necessary to compare the results of the 1944-45 season's results with those reported previously (1) rather than with those of the last two seasons.

Two soil types were used in the work of the last three seasons listed above, the medium sand of the Tobacco Research Station and a silt loam soil of a commercial grower. Both areas were sufficiently near to one another for the rainfall conditions to be considered the same. At the Research Station the same plot of ground was planted for these experiments in the three seasons. On the grower's soil a different area, damper and likely to be colder in the spring, was used in the last two seasons from that set out in 1944-45. Plants in each season were obtained from the same seedling bed and were set out in the field as nearly as possible on the same day. The planting dates for the three seasons were 24th November, 1944, 3rd December, 1945 and 3rd December, 1946. Fertilizer equivalent to 1,000 lb. per acre of a 3-8-8 mixture was applied in the rows before planting. Spacing of the plants was 24 in. in rows 3 ft. 6 in. apart. The usual cultural operations were given, including irrigation when required on the medium sand.

The number of plants taken as a sample at any one date varied with their size, but was never less than ten, even with fully grown plants. Roots were not included, the plants being cut off at ground level. Separations into leaves and stalks were made at each sampling until harvesting began. From then onwards separations were made into ripe and green leaves, laterals and stalks in the 1944-45 seasons, and into ripe, nearly ripe and green leaves, toppings, laterals and stalks in the 1945-46 and 1946-47 seasons. At each harvest usually three leaves were taken in the " ripe " leaf sample ; similarly three leaves were taken for the " nearly ripe " leaf sample, the assumption being that these " nearly ripe " leaves would be the " ripe " leaves at the next harvest. Also the number of leaves on an average plant was recorded after topping. At subsequent sampling dates only average-sized plants with the requisite number of leaves were taken for sample material. By this means it was expected that more accurate sampling would be obtained. Ripe leaf from the area at the Research Station was picked by the Station staff after the sample plants were obtained. This leaf was cured and provided material for analysis representing the successive harvests from the plants. The same number of leaves per plant was removed for these harvests as was taken as " ripe " on the plants for laboratory examination. Cured leaf was analysed for minerals and nitrogen contents, as were the other separates of plant material, and also for glucose, fructose and sucrose contents.

Monthly totals of rainfall for the three seasons under review are given in Table I and a more detailed record of the distribution of the rainfall by weekly periods in Fig. 1. The 1944-45 season was wet in the spring and during the harvesting period of January and February. November and December were not unduly wet. Temperatures and hours of bright sunshine were low in the spring and early summer. No irrigation water was applied in this season. In 1945-46 the spring rainfall was low and this lack of rain continued into the growing and harvesting season. Most of the December rainfall occurred in the first ten days of the month. Both January and February were unusually dry, the latter month providing only 0.56 in. of rain. Over an inch of rain fell in the first few days of March and further rain fell later. This, combined with the previous very dry period, caused unsatisfactory harvesting conditions. Some relief from the dry conditions of January was obtained on the medium sand by applying irrigation water equivalent to approximately one inch of rain on each of two occasions, namely 15th and 30th January, 1946. In the third season there was a sufficiency of soil moisture up to November, 1946. Most of the rain recorded fell before the middle of the month. From then onwards there were long dry periods in December, January, February and March. Up to the end of December the season was unusually cold but later the weather was warm and sunny, causing a rapid ripening of the leaf. The crop was irrigated at the previously mentioned rate on 29th January, 1947.

TABLE I RAINFALL IN INCHES AT THE TOBACCO RESEARCH STATION FOR THE SEASONS 1944-45, 1945-46, 1946-47 AT MONTHLY INTERVALS

Season	Sept	Oct	Nov	Dec	Jan	Feb	March	April.	Total
1944-45	3.16	8.65	2.44	3.57	5.05	8.35	2.09	2.32	35.6
1945-46	3.83	3.45	1.71	2.96	1.13	0.56	3.73	8.71	26.0
1946-47	5.47	8.04	2.27	1.35	1.93	1.94	2.19	8.29	31.4

A. 1944-45 SEASON

In this season the same strain of Harrison's Special variety was grown as in the 1941-42 and 1942-43 trials. Owing to the unusually wet spring and low temperatures early in the season the difference between the rates of growth of the plants on the two areas was greater than might otherwise have been the case. During January and February when the leaf was maturing there were some very heavy falls of rain (Table I and Fig. 1). As already stated it was unnecessary to irrigate the plants at the Research Station.

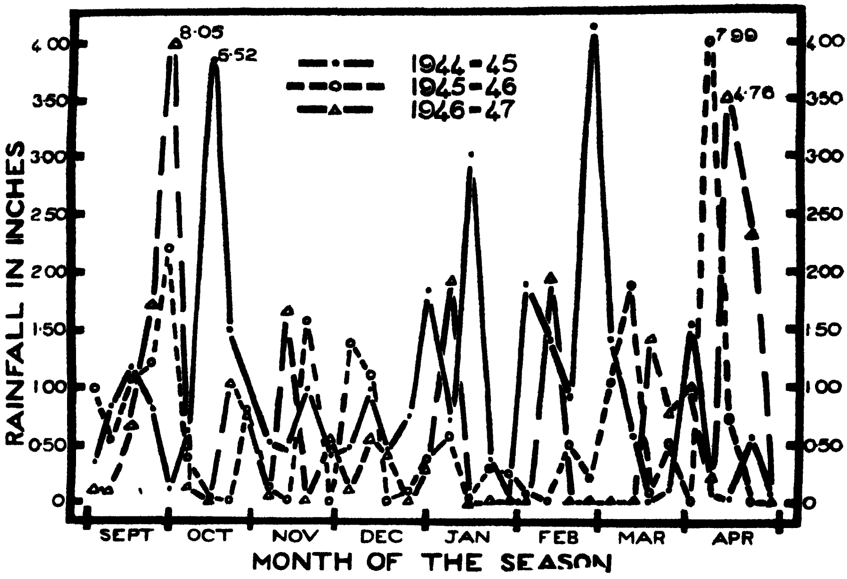

FIG. 1.—Rainfall at weekly intervals for the seasons 1944-45, 1945-46 and 1946-47

Yield of Dry Matter :

Data for yield of dry matter calculated in terms of grams per plant and pounds per acre and for percentage of dry matter in the leaves and stalks are given in Table II for the Research Station trial. After setting of the plants in the field on 24th November, 1944, no samples were taken until 10th January, 1945, i.e., 47 days after planting, the plants then being about 15 in. high. At this stage the leaves provided about seven-eighths of the total dry matter of the plants. Percentage of dry matter was 10.52 per cent. in the leaves and only 7.13 per cent. in the stalks, with a plant average of 9.92 per cent. The average dry matter per plant was 27.38 g. In the next few weeks the plants grew rapidly, with increasing percentages of dry matter in both leaves and stalks ; moreover the stalks now formed a larger proportion of the total weight of the plant. At the time of the first harvest on 31st January, 1945, 68 days after planting, the plants were about 45 inches high and were yellowing on account of the dry weather. The stalks comprised almost one-third of the total weight of the plant while the average weight of dry matter per plant had increased to 108.6 g. Moreover the separation made into sand leaves, ripe leaves and green leaves showed that the percentage of dry matter increased in this order, the stalks having an appreciably lower percentage. After harvesting began the amount of leafage on the plants decreased from one date of sampling to the next, but the stalks continued to increase in weight until the end of the season. The last harvest was made on 6th March, 102 days after setting of the plants in the field.

TABLE II. NUTRIENT INTAKE EXPERIMENT 1944-45 SEASON, TOBACCO RESEARCH STATION, MEDIUM SAND SOIL.
Yield of Dry Matter and Percentage of Dry Matter

Date of Sampling	Part of Plant.	Dry Matter per cent	Dry Matter per plant gm	Dry Matter per acre lb.
24/11/44	Whole	5.16	0.43	5.65
10/1/45	Leaves	10.52	23.93	316.5
	Stalks	7.13	3.45	45.6
	Whole	9.92	27.38	362.1
17/1/45	Leaves	14.79	39.34	520.3
	Stalks	7.59	8.68	114.8
	Whole	12.63	48.02	635.1
24/1/45	Leaves	14.09	57.07	754.9
	Stalks	9.51	19.14	253.1
	Whole	12.57	76.21	1008.0
31/1/45	Sand Leaves	13.50	10.36	137.0
	Ripe Leaves	14.12	19.03	251.7
	Green leaves	18.92	43.28	572.5
	Stalks	12.02	35.90	474.8
	Whole	14.69	108.57	1436.0
7/2/45	2 ripe leaves	16.89	13.45	177.9
	Green leaves	18.57	41.51	549.1
	Toppings leaves	18.19	6.75	89.3
	Toppings stalks	11.94	11.46	151.6
	Stalks	14.49	48.09	636.1
	Whole	15.79	121.26	1604.0
14/2/45	2 ripe leaves	19.03	14.90	197.1
	7 green leaves	19.34	30.80	407.4
	Stalks	15.71	53.97	713.9
	Whole	17.15	99.67	1318.4
27/2/45	3 ripe leaves	17.46	18.72	247.6
	4 green leaves	17.54	18.56	245.5
	Laterals	12.82	21.04	278.3
	Stalks	15.39	49.34	652.6
	Whole	15.43	107.66	1424.0
6/3/45	4 ripe leaves	18.23	16.58	219.3
	Laterals	12.54	23.11	305.7
	Stalks	17.70	60.95	806.2
	Whole	16.24	100.64	1331.2

For the silt loam soil data corresponding to those given above for the Research Station are presented in Table III. Samples were taken mostly on the same dates from both areas; direct comparisons of the development of the plants are therefore readily obtained. During the first 68 days after planting the yield of dry matter per plant was lower on the silt loam than on the medium sand, but from this date onward the plants on the heavier soil rapidly increased in weight due especially to the development of very heavy stalks. Thus by the end of the season the stalks of these plants were fifty per cent. greater in size than those on the lighter soil. During most of the season the plants on the silt loam showed percentages of dry matter in the leaves lower by from three to nearly five per cent. than those for corresponding dates on the medium sand. Stalks showed similar, though smaller, differences. At the end of the season the stalks showed little difference but the leaves from the plants on the two areas still were markedly different in dry matter content.

TABLE III. NUTRIENT INTAKE EXPERIMENT 1944-45 SEASON, LIGHT-PHASE SILT-LOAM SOIL

Yield of Dry Matter and Percentage of Dry Matter

Date of Sampling.	Part of Plant	Dry Matter per cent.	Dry Matter per plant gm.	Dry Matter per acre lb.
24/11/44	Whole	5.16	0.43	5.65
10/1/45	Leaves	7.51	16.16	213.7
	Stalks	6.82	2.66	35.2
	Whole	7.40	18.82	248.9
17/1/45	Leaves	10.30	34.78	460.0
	Stalks	7.42	6.63	87.7
	Whole	9.70	41.41	547.7
24/1/45	Leaves	10.87	51.78	684.9
	Stalks	8.25	17.95	237.4
	Whole	10.05	69.73	922.3
31/1/45	4 Ripe leaves	13.55	24.85	328.7
	Green leaves	16.22	36.86	487.5
	Laterals and sand leaves	11.42	18.83	249.1
	Stalks	10.68	30.59	404.6
	Whole	12.89	111.13	1469.9
7/2/45	4 Bottom leaves	13.09	31.10	411.4
	Leaves	16.26	52.87	699.3
	Stalks	12.33	55.16	729.6
	Whole	13.77	139.13	1840.3
14/2/45	4 Bottom Leaves	13.82	28.48	376.7
	Leaves	16.08	71.31	943.2
	Stalks	14.38	69.35	917.3
	Whole	14.94	169.14	2237.2
22/2/45	3 Ripe leaves	11.99	16.72	221.2
	Leaves	15.89	86.21	1140.3
	Laterals	10.54	16.96	224.3
	Stalks	15.26	78.22	1034.6
	Whole	14.62	198.11	2620.4
6/3/45	3 Ripe leaves	12.06	16.40	216.9
	Leaves	14.51	70.92	938.1
	Laterals	11.76	44.86	593.3
	Stalks	18.02	95.53	1263.6
	Whole	14.82	227.71	3011.9

A comparison of the yields of dry matter for these two areas clearly shows the greater development of the plants on the heavier soil. The full season's figures were not obtained for plants on the latter area because harvesting ceased on 6th March. At this time the plants were obviously still growing rapidly, whereas at this time the Research Station area had been completely harvested. It is of interest to note that the heavier soil produced nearly twice as much dry matter as the lighter one.

Chemical Composition of Plant Separates :

Determinations of the mineral and nitrogen contents of leaves and stalks from the Research Station are set out in Table IV, the figures given being calculated on a sand-free dry-matter basis. Early in the season the plants were rich in all the constituents determined, this being especially marked in the case of potash. The maximum figures for leaves and stalks, 47 days after planting were respectively 6.08 per cent. and 7.86 per cent. As the plants developed there was a marked tendency for the percentages of magnesia, phosphoric acid, potash and nitrogen to fall. On the other hand lime increased in the lowest leaves to a maximum of 5.13 per cent. CaO ; leaves higher up the plant were poorer in lime, even though there was a tendency for leaves near the top of the plants to show an increase in lime content. Stalks progressively became poorer in all constituents until the harvest on the 82nd day after planting, after which they remained essentially of a constant composition, this being soluble ash, 6.7 per cent., lime, 0.9 per cent., magnesia, 0.3 per cent., phosphoric acid, 0.4 per cent., potash, 3.1 per cent. and nitrogen, 1.0 per cent.

TABLE IV CHEMICAL COMPOSITION OF TOBACCO, NUTRIENT INTAKE EXPERIMENT, TOBACCO RESEARCH STATION 1944-45 SEASON
Expressed as percentage on sand-free dry matter basis

Date of Sampling	Part of Plant	Insoluble Ash	Soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N
24/11/44	Whole	0.11	15.09	2.79	0.78	0.97	6.08	2.68
10/1/45	Leaves	0.65	17.82	3.66	1.08	0.76	6.08	3.47
	Stalks	0.11	15.66	1.36	0.58	0.75	7.86	2.45
17/1/45	Leaves	0.57	14.85	3.22	0.87	0.66	5.29	2.83
	Stalks	0.04	13.36	1.30	0.50	0.58	6.59	1.87
24/1/45	Leaves	0.37	11.72	2.48	0.64	0.59	4.29	1.92
	Stalks	0.10	11.52	1.12	0.46	0.59	5.88	1.41
31/1/45	Sand Leaves	0.97	17.81	5.13	1.10	0.48	3.63	1.30
	Ripe leaves	0.71	13.50	3.40	0.75	0.49	4.53	1.33
	Green leaves	0.19	8.90	1.55	0.42	0.60	3.72	1.76
	Stalks	0.09	8.87	1.01	0.40	0.55	4.34	1.17
7/2/45	2 Ripe leaves	0.19	10.10	2.65	0.40	0.62	3.37	1.56
	Green leaves	0.15	9.03	1.85	0.43	0.61	3.60	1.94
	Top leaves	0.01	10.05	1.74	0.54	0.94	4.45	3.31
	Toppings	0.00	10.65	1.04	0.61	1.07	5.22	2.72
	Stalks	0.03	7.74	1.01	0.37	0.47	3.80	0.99
14/2/45	2 Ripe leaves	0.08	9.46	2.28	0.48	0.54	3.53	1.66
	7 Green leaves	0.00	9.45	2.10	0.48	0.59	3.81	2.17
	Stalks	0.01	6.57	0.89	0.31	0.45	3.07	1.03
27/2/45	3 Ripe leaves	0.03	11.32	2.84	0.78	0.61	3.90	2.44
	4 Green leaves	0.08	11.95	3.08	0.91	0.55	3.64	2.81
	Laterals	0.07	20.15	2.26	0.89	0.87	5.08	3.61
	Stalks	0.17	7.08	0.98	0.33	0.38	3.29	1.05
6/3/45	4 Ripe leaves	0.03	12.29	3.47	0.89	0.68	3.54	2.42
	Laterals	0.00	14.47	2.74	0.93	0.88	5.55	3.49
	Stalks	0.08	6.40	0.95	0.32	0.34	2.92	0.97

Chemical data for plants from the silt loam soil are given in Table V, where it will be seen that in the early samplings the leaves and stalks were very similar in composition to corresponding material from the Research Station plants grown on a medium sand. As the plants developed some significant differences appeared. Thus on the heavier soil the soluble ash and nitrogen were present in greater concentration and the lime contents of the leaves taken towards the end of the season were very high, the maximum figure being 8.25 per cent. CaO. Potash contents were similar for each corresponding set from the two areas. It is interesting to note the very high potash content, 5 per cent. K_2O or more, of the young laterals in both series of samples. In general there were no very consistent differences between the concentrations of magnesia and phosphoric acid in the two sets of data.

TABLE V CHEMICAL COMPOSITION OF TOBACCO, NUTRIENT INTAKE EXPERIMENT, LIGHT-PHASE SILT-LOAM SOIL, 1944-45 SEASON

Expressed as percentage on sand-free dry matter basis

Date of Sampling	Part of Plant.	Insoluble Ash	Soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N
24/11/44	Whole	0.11	15.09	2.79	0.78	0.97	6.08	2.68
10/1/45	Leaves	0.29	17.66	3.62	0.75	0.79	6.68	4.18
	Stalks	0.12	15.87	1.46	0.52	0.83	7.91	3.04
17/1/45	Leaves	0.09	17.24	3.94	0.70	0.72	6.25	4.04
	Stalks	0.18	14.38	1.68	0.48	0.66	7.10	2.52
24/1/45	Leaves	0.23	15.14	3.65	0.64	0.71	5.26	3.28
	Stalks	0.09	12.43	1.37	0.41	0.62	6.09	2.15
31/1/45	4 Ripe leaves	0.61	14.58	3.97	0.47	0.47	4.68	2.22
	Green leaves	0.03	9.49	2.04	0.34	0.68	3.78	2.95
	Laterals and sand leaves	0.65	17.65	4.87	0.69	0.53	5.21	2.51
	Stalks	0.04	9.43	1.22	0.27	0.59	4.50	1.71
7/2/45	4 Bottom leaves	0.18	16.02	4.99	0.57	0.43	4.38	2.24
	Leaves	0.05	11.22	2.97	0.37	0.53	3.87	2.79
	Stalks	0.07	9.16	1.38	0.32	0.46	4.22	1.61
14/2/45	4 Bottom leaves	0.34	16.60	5.64	0.60	0.44	4.08	2.01
	Leaves	0.16	12.30	3.63	0.43	0.55	3.78	2.80
	Stalks	0.06	7.22	1.24	0.29	0.44	2.15	1.46
22/2/45	3 Ripe leaves	0.40	19.46	6.85	0.74	0.45	4.15	2.03
	Green leaves	0.09	13.10	4.20	0.45	0.51	2.96	2.53
	Laterals	0.04	12.64	2.02	0.54	1.07	5.39	4.07
	Stalks	0.02	6.83	1.23	0.32	0.39	3.03	1.32
6/3/45	3 Ripe leaves	0.04	22.02	8.25	0.86	0.41	3.82	2.25
	Leaves	0.11	15.40	5.18	0.56	0.48	2.74	2.75
	Laterals	0.15	11.80	1.96	0.50	0.80	4.67	3.42
	Stalks	0.02	5.95	1.08	0.25	0.30	1.74	1.16

Intake of Nutrients:

From the yield of dry matter and the chemical composition of the plants the intake of nutrients is readily calculated. Nutrient status of the plants at the two locations is given in Tables VI and VII. In the earlier stages of growth the greatest demand was for potash, lime and nitrogen, the demand decreasing in this order. At all stages the demand for potash was double that for lime or nitrogen. Uptake of magnesia and phosphoric acid was at a much lower level.

TABLE VI NUTRIENT STATUS ON PER PLANT BASIS, MEDIUM SAND SOIL, TOBACCO RESEARCH STATION, 1944-45 SEASON

Expressed in grams on sand-free dry matter basis

Date of Sampling.	Part of Plant.	In-soluble Ash	Soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N
24/11/44	Whole	0.001	0.064	0.012	0.003	0.004	0.025	0.011
10/1/45	Leaves	0.16	4.26	0.88	0.26	0.18	1.45	0.83
	Stalks	0.004	0.54	0.05	0.02	0.03	0.27	0.08
	Whole	0.164	4.80	0.93	0.28	0.21	1.72	0.91
17/1/45	Leaves	0.22	5.84	1.27	0.34	0.26	2.08	1.11
	Stalks	0.003	1.16	0.11	0.04	0.05	0.57	0.16
	Whole	0.223	7.00	1.38	0.38	0.31	2.65	1.27
24/1/45	Leaves	0.21	6.69	1.42	0.37	0.34	2.45	1.10
	Stalks	0.02	2.20	0.21	0.09	0.11	1.13	0.27
	Whole	0.23	8.89	1.63	0.46	0.45	3.58	1.37
31/1/45	Sand leaves	0.09	1.85	0.53	0.11	0.05	0.38	0.13
	Ripe leaves	0.13	2.57	0.65	0.14	0.09	0.86	0.25
	Green leaves	0.08	3.85	0.67	0.18	0.26	1.61	0.76
	Stalks	0.04	3.18	0.36	0.14	0.20	1.56	0.42
	Whole	0.34	11.45	2.21	0.57	0.60	4.41	1.56
7/2/45	2 Ripe leaves	0.02	1.36	0.36	0.05	0.08	0.45	0.21
	Green leaves	0.06	3.75	0.77	0.18	0.25	1.49	0.81
	Top leaves	0.00	0.68	0.12	0.04	0.06	0.30	0.22
	Toppings	0.00	1.22	0.12	0.07	0.12	0.60	0.31
	Stalks	0.02	3.72	0.49	0.18	0.23	1.83	0.48
	Whole	0.10	10.73	1.86	0.52	0.74	4.67	2.03
14/2/45	2 Ripe leaves	0.01	1.41	0.34	0.07	0.08	0.53	0.25
	7 Green leaves	0.00	2.91	0.65	0.15	0.18	1.17	0.67
	Stalks	0.005	3.55	0.48	0.17	0.24	1.66	0.56
	Whole	0.015	7.87	1.47	0.39	0.50	3.36	1.48
27/2/45	3 Ripe leaves	0.006	2.12	0.53	0.15	0.11	0.73	0.46
	4 Green leaves	0.01	2.22	0.57	0.17	0.10	0.68	0.52
	Laterals	0.01	4.24	0.48	0.19	0.18	1.07	0.76
	Stalks	0.09	3.49	0.48	0.16	0.19	1.62	0.52
	Whole	0.116	12.07	2.06	0.67	0.58	4.10	2.26
6/3/45	4 Ripe leaves	0.005	2.04	0.58	0.15	0.11	0.59	0.40
	Laterals	0.00	3.34	0.63	0.21	0.20	1.28	0.81
	Stalks	0.05	3.90	0.58	0.20	0.21	1.78	0.59
	Whole	0.055	9.28	1.79	0.56	0.52	3.65	1.80

TABLE VII. NUTRIENT STATUS ON PER PLANT BASIS, LIGHT-PHASE SILT-LOAM SOIL, 1944-45 SEASON

Expressed in grams on sand-free dry matter basis

Date of Sampling	Part of Plant.	Insoluble Ash.	Soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5.	Potash K_2O	Total Nitrogen N
24/11/44	Whole	0.001	0.064	0.012	0.003	0.004	0.025	0.011
10/1/45	Leaves	0.05	2.85	0.58	0.12	0.13	1.08	0.68
	Stalks	0.01	0.42	0.04	0.01	0.02	0.21	0.08
	Whole	0.06	3.27	0.62	0.13	0.15	1.29	0.76
17/1/45	Leaves	0.03	6.00	1.37	0.24	0.25	2.17	1.41
	Stalks	0.01	0.95	0.11	0.03	0.04	0.47	0.17
	Whole	0.04	6.95	1.48	0.27	0.29	2.64	1.58
24/1/45	Leaves	0.12	7.84	1.89	0.33	0.37	2.72	1.70
	Stalks	0.02	2.23	0.25	0.07	0.11	1.09	0.39
	Whole	0.14	10.07	2.14	0.40	0.48	3.81	2.09
31/1/45	4 Ripe leaves	0.15	3.62	0.99	0.12	0.12	1.16	0.55
	Green leaves	0.01	3.50	0.75	0.13	0.25	1.39	1.09
	Laterals and sand leaves	0.13	3.32	0.92	0.13	0.10	0.98	0.47
	Stalks	0.02	2.88	0.37	0.08	0.18	1.38	0.52
	Whole	0.31	13.32	3.05	0.46	0.65	4.91	2.63
7/2/45	4 Bottom leaves	0.06	4.98	1.55	0.18	0.13	1.36	0.70
	Leaves	0.03	5.93	1.57	0.20	0.28	2.05	1.48
	Stalks	0.04	5.05	0.76	0.18	0.25	2.33	0.89
	Whole	0.13	15.96	3.88	0.56	0.66	5.74	3.07
14/2/45	4 Bottom leaves	0.09	4.73	1.61	0.17	0.13	1.16	0.57
	Leaves	0.12	8.77	2.59	0.31	0.39	2.70	2.00
	Stalks	0.04	5.01	0.86	0.20	0.31	1.49	1.01
	Whole	0.25	18.51	5.06	0.68	0.83	5.35	3.58
22/2/45	3 Ripe leaves	0.07	3.25	1.15	0.12	0.08	0.69	0.34
	Green leaves	0.08	11.29	3.62	0.39	0.44	2.55	2.18
	Laterals	0.01	2.14	0.34	0.09	0.18	0.91	0.69
	Stalks	0.02	5.34	0.96	0.25	0.30	2.37	1.03
	Whole	0.18	22.02	6.07	0.85	1.00	6.52	4.24
6/3/45	3 Ripe leaves	0.01	3.61	1.35	0.14	0.07	0.63	0.37
	Leaves	0.08	10.92	3.67	0.40	0.34	1.94	1.95
	Laterals	0.07	5.29	0.88	0.22	0.36	2.09	1.53
	Stalks	0.02	5.68	1.03	0.24	0.29	1.66	1.11
	Whole	0.18	25.50	6.93	1.00	1.06	6.32	4.96

While the data on the per plant basis are of more interest to the investigator than corresponding figures on a per acre basis, the latter have a value to the agriculturist in showing the demand on the soil for nutrients provided from its own resources or from those in conjunction with added fertilizer. By using the factor 13.227 data on the gram per plant basis may be calculated to a pounds per acre basis, assuming that 6,000 plants were set out per acre. Nutrient status of the whole plants for the various sampling periods is given below in Table VIII, the quantities of each nutrient being expressed in pounds per acre.

TABLE VIII NUTRIENT STATUS OF PLANTS, IN POUNDS PER ACRE, 1944-45

Location.	Date of Sampling.	Soluble Ash lb	Lime CaO lb	Magnesia MgO lb	Phosphoric Acid P_2O_5 lb	Potash K_2O lb	Nitrogen N lb
Medium sand	24/11/44*	0.86	0.16	0.04	0.05	0.33	0.15
	10/1/45	63.5	12.3	3.7	2.8	22.8	12.0
	17/1/45	92.6	18.3	5.0	4.1	35.1	16.8
	24/1/45	117.6	21.6	6.1	6.0	47.4	18.1
	31/1/45	151.4	29.2	7.5	7.9	58.3	20.6
	7/2/45	141.9	24.6	6.9	9.8	61.8	26.9
	14/2/45	104.1	19.4	5.2	6.6	44.5	19.6
	27/2/45	159.6	27.3	8.9	7.7	54.2	29.9
	6/3/45	122.8	23.7	7.4	6.9	48.3	23.8
Silt loam	24/11/44*	0.86	0.16	0.04	0.05	0.33	0.15
	10/1/45	43.3	8.2	1.7	2.0	17.1	10.0
	17/1/45	91.9	19.6	3.6	3.8	34.9	20.9
	24/1/45	133.2	28.3	5.3	6.3	50.4	27.7
	31/1/45	176.2	40.0	6.1	8.6	64.9	34.8
	7/2/45	211.1	51.3	7.4	8.7	75.9	40.6
	14/2/45	244.8	66.9	9.0	11.0	70.8	47.4
	22/2/45	291.3	80.3	11.3	13.3	86.2	56.1
	6/3/45	337.3	91.7	13.2	14.0	83.6	65.6

* Date of planting in the field

The data of Table VIII show that during the period of most rapid growth at the Research Station the plants were accumulating potash at the rate of about 1 lb of K_2O per acre per day and nitrogen and lime at about half this rate. Magnesia and phosphoric acid were being absorbed at the rate of only 0.2 lb. per acre per day. But on the heavier soil potash was being taken up at the rate of more than 2 lb. per acre per day. Nitrogen and lime rates were 1-1½ lb. per day. Magnesia and phosphoric acid were being absorbed at about the same or slightly lower rate than at the Research Station. Once harvesting began on 31st January at the Research Station the state of affairs on the two areas was quite different. Due to removal of ripe leaves and a lower rate of absorption the quantity of nutrients in later samples from the Research Station was generally lower than at 31st January, but on the silt loam accumulation of nutrients proceeded at rates as great as or greater than those in the earlier period. This was due to the continued rapid development of dry matter on this area. Harvesting did not begin here until 22nd February.

Discussion :

Analysis of the detailed data for periods after harvesting began shows that ripe leaves removed at any one harvest had a lower percentage of dry matter than the remaining leaves but that in passing towards the top of the plant there was a tendency for the ripe leaves to approach more closely to the dry matter content of the remainder. This difference in composition was particularly well-marked on the heavier soil.

Examination of the data for development of dry matter after harvesting began at the Research Station shows that increases, as set out in Table IX, occurred during the periods between the first and the second dates of each line of the table, these increases being calculated from the total weight of remaining leaves and stalk, after removal of ripe leaves on the first date, but including ripe leaves on the second date. It is clear that this should be done because " nearly ripe " leaves of the first date of any pair will become the " ripe " leaves picked on the second date.

TABLE IX. INCREMENTS OF DRY MATTER IN POUNDS PER ACRE BETWEEN SUCCESSIVE SAMPLINGS

Location.	Period.	Rainfall in	Total Dry Matter Increment lb.	Leaf Dry Matter Increment lb.
Medium sand	31/1/45-7/2/45	1.85	556.7	243.8
	7/2/45-14/2/45	1.45	133.2	55.4
	14/2/45-27/2/45	0.89	302.7	85 7
	27/2/45-6/3/45	4.50	154.8	26.2
	Totals	8.69	1147.4	411.1
Silt loam	31/1/45-7/2/45	1.85	370.4	294.5
	7/2/45-14/2/45	1.45	396.9	209.2
	14/2/45-22/2/45	0.89	383.2	41.6
	22/2/45-6/3/45	4.50	391.5	15.3
	Totals	8.69	1542.0	560.6

These data demonstrate plainly the continued growth of stalk and laterals at the Research Station (medium sand) as compared with the development of harvestable leaves, the increment for harvestable leaves falling rapidly after 7th February. In other words, leaves left on the plant for later harvests did not grow very much in the time intervening between one harvest and the next. Doubtless the dry weather in February reduced the rate of growth not only of harvestable leaves but of the plants as a whole. On the silt loam rapid growth continued during February until the first harvest on the 22nd. These plants had been topped at about four feet high about 10 days previously. After the first harvest was taken there was a very marked drop in dry matter increment of the leaves. Rain came too late for it to have a marked effect on growth before 6th March, the date of the last recorded figures. Stalks and laterals continued to develop rapidly even though leaf growth was restricted.

Changes in chemical composition of the leaves and other parts of the plants occurred also after harvesting began. On the medium sand lime, magnesia and potash decreased in the leaves during ripening until about the middle of the plant was reached (third harvest). The two former constituents then increased with increasing maturity of the leaves but potash tended to decrease slightly. Phosphoric acid and nitrogen increased in percentage in passing from lower to upper leaves ; the former tended to increase but the latter to decrease during maturation of the leaves. On the heavier soil lime and magnesia increased in concentration in leaves nearer the top of the plant and also increased during maturation. Phosphoric acid and nitrogen decreased in percentage

TABLE X CHEMICAL COMPOSITION OF CURED LEAF FROM SUCCESSIVE HARVESTS, TOBACCO RESEARCH STATION, 1944-45 SEASON

Constituents expressed as percentage on sand-free dry matter basis

Date of Sampling.	Soluble Ash.	In soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N	Glucose	Fructose	Sucrose.	Total Sugars	Ratio, Total Sugars : Total Nitrogen
Blades												
31/1/45	11.11	0.18	3.64	0.68	0.40	2.74	1.41	13.51	14.88	5.99	34.38	24.4
7/2/45	9.89	0.22	3.04	0.51	0.49	2.78	1.86	15.18	12.94	4.49	32.61	17.5
14/2/45	10.27	0.26	2.91	0.49	0.56	2.66	2.04	14.04	11.22	0.00	25.26	12.4
1/3/45	10.49	0.16	3.11	0.64	0.61	2.47	2.67	9.23	7.48	1.80	18.51	6.9
7/3/45	12.72	0.33	4.08	0.84	0.53	2.97	2.89	7.86	5.70	0.98	14.54	5.0
Midribs												
31/1/45	17.77	0.19	3.41	0.87	0.50	6.47	1.27	9.83	9.26	4.76	23.85	18.8
7/2/45	15.67	0.17	2.78	0.65	0.62	6.47	1.37	12.66	7.13	2.77	22.56	16.5
*14/2/45	15.05	0.13	2.89	0.78	0.83	5.53	1.94	10.39	4.04	n.d.		-
1/3/45	16.71	0.17	3.31	1.11	0.95	5.77	1.92	7.86	3.25	1.05	12.16	6.3
*7/3/45	17.86	0.19	3.53	1.14	0.79	6.31	1.31	6.06	2.35	0.52	8.93	6.8

* Samples overheated in drying oven

amount during ripening and were relatively constant in passing from lower to upper leaves. Potash tended to decrease towards the top of the plant, but to increase during maturation of the leaves; the content of potash remained relatively constant however at about 4 per cent. K_2O.

From the detailed data on the amounts of the several nutrients in the ripe and unripe leaves and in the stalks and laterals it is demonstrable that right through the season, even after harvesting began, accumulation of nutrients still took place, often to an appreciable extent, although of course the plants were carrying fewer leaves after each succeeding harvest. This was true for the total intake at both locations. From the more complete data for the medium sand area it is clear that nutrients continued to pass into the leaves during ripening. For lime, magnesia and phosphoric acid the intake by the plant after harvesting began comprised for each of these constituents approximately half the intake for the whole season; for soluble ash and potash the proportion was two-fifths and for nitrogen three-fifths. Thus in the latter case more nutrient was absorbed after harvesting began than in the earlier portion of the season. On a daily basis the increments of nutrient for magnesia and phosphoric acid averaged about 0.2 lb. per acre per day but for lime, potash and nitrogen the average was about one pound per acre per day. Over corresponding periods on the silt loam, with the continued rapid growth, the rate of intake reached double that for the medium sand.

On each occasion that ripe leaves were available the experimental area was picked over by the Research Station staff after the whole plants had been cut for analytical work. These leaves were flue-cured in the same manner as the crop of the Station. Each harvest was kept separate and samples of cured leaf were made available for analysis at the end of the season. This material was examined for minerals, nitrogen and sugars contents. The leaves having been separated into blade and midrib portions renders it impossible to make a direct comparison with the minerals and nitrogen data for the ripe (whole) leaves from the plants selected in the field. Analyses of cured leaf separates, blade and midrib for each of the five harvests of the 1944-45 season are given in Table X.

Taking, first, comparison of blade with midrib, it is noted that the latter is outstandingly rich in potash, there being more than twice as much in the midrib as in the blade. The midrib is also very rich in soluble ash. Lime content is approximately the same in both portions of the leaves but magnesia and phosphoric acid are present in greater amounts in the midribs; on the other hand nitrogen is usually appreciably greater in the blade. In respect of changes with successive harvests, soluble ash, lime, magnesia and potash show higher figures for early and late harvests than for intermediate ones. Phosphoric acid and nitrogen tend to increase in passing from lower to upper leaves; the last sample however appears to be aberrant in the midrib for these constituents.

Glucose, fructose and sucrose are higher in the blade than in the midrib. Two samples of midrib were unfortunately apparently overheated during drying in the laboratory so that the sugars data for these are somewhat doubtful. High figures for sugars are shown for the first three sets of samples. There is a marked tendency for sugar content to fall, especially in respect to sucrose, after the second harvest.

This combined with increasingly large nitrogen contents, especially in the blade, results in the ratio of total sugars to total nitrogen showing a marked decline with each successive harvest. The ratios for later harvests are such that the leaf is indicated to be of poor quality for cigarette purposes.

B. 1945-46 AND 1946-47 SEASONS

It has already been stated above that in these two seasons a different, broader leaved strain of Harrison's Special variety of tobacco was used than the one previously grown at the Research Station. Moreover a comparison of development of the plant and nutrient status has been made on two neighbouring soil types. More detailed records have been kept also during the experiments than previously.

In both seasons the plants were set out on 3rd December. The 1945-46 season was an exceptionally dry one and irrigation water was supplied on 15th and 30th January, 1946, at the Research Station. On the heavier soil of the commercial grower's property no supplementary water was used. Over most of the growing and harvesting period in 1946-47 the weather was dry. At the Research Station the crop was irrigated on 29th January, 1947. No water was supplied to the crop on the heavier soil.

YIELD OF DRY MATTER

(a) *Medium Sand (Tobacco Research Station):*

Yield of dry matter in terms of grams per plant and pounds per acre, and percentage of dry matter, in the various separates from the plants at the Tobacco Research Station in the 1945-46 season are shown in Table XI. It will be noted that the young plants were very high in dry matter content as compared with previous seasons. At the first sampling after setting in the field (at 44 days on 16th January, 1946) the percentage of dry matter in the leaves was still relatively high but that of the stalks had fallen to a level near that previously recorded for young plants. Growth had been fairly satisfactory in spite of the rather cold but fairly dry December. No doubt the warmth and dryness of January had brought the plants ahead. At this time they were 12 14 in high and had 7 formed leaves. The weekly samplings show that rapid growth continued through January and February. Thus between 16th January and 23rd January the plants doubled in height. By the end of the month they were sending up flower stalks. When the first harvest of ripe leaves was made on 11th February the average dry matter per plant was 185.47 g., this being equivalent to 2453.2 lb. per acre. The dry matter content of the ripe leaves was 15.59 per cent. with unripe leaves showing nearly 19 per cent. Maximum yield of dry matter in leaves was reached at the sampling on 25th February, in spite of the previous removal of six ripe leaves from the plant. By this time too the stalks had almost reached their greatest weight. In keeping with the dry conditions the percentages of dry matter in the leaves at the end of February and in March were very high, the maximum figures being 23.0 per cent. for ripe and 25.9 per cent. for nearly ripe leaves. It is noticeable that on every occasion ripe leaves showed a percentage of dry matter, usually lower by about one per cent., than that of nearly

TABLE XI. NUTRIENT INTAKE EXPERIMENT 1945-46 SEASON, TOBACCO RESEARCH STATION, MEDIUM SAND SOIL

Yield of Dry Matter and Percentage of Dry Matter

Date of Sampling	Part of Plant.	Dry Matter per cent	Dry Matter per plant. gm	Dry Matter per acre. lb.
3/12/45	Whole	24.32	0.98	13.0
16/1/46	Leaves	14.94	29.81	394.3
	Stalks	8.74	4.53	59.9
	Whole	13.66	34.34	454.2
23/1/46	Leaves	13.76	52.57	695.3
	Stalks	8.34	11.12	147.1
	Whole	12.33	63.69	842.4
30/1/46	Leaves	16.22	81.26	1074.8
	Stalks	9.41	22.50	297.6
	Whole	14.00	103.76	1372.4
6/2/46	Leaves	17.88	92.71	1226.3
	Stalks	11.97	46.44	614.3
	Whole	15.35	139.15	1840.6
11/2/46	3 Ripe Leaves	15.59	20.53	271.5
	3 Near ripe leaves	18.98	29.77	393.8
	13 Green leaves	18.76	65.54	866.9
	Stalks and flower head	14.76	69.63	921.0
	Whole	16.71	185.47	2453.2
18/2/46	3 Ripe leaves	17.04	25.57	338.2
	3 Near ripe leaves	17.50	32.39	428.4
	10 Green leaves	20.80	57.10	755.3
	Toppings (13/2/46)	15.82	10.57	139.8
	Stalks	13.96	67.73	895.9
	Whole	16.64	193.36	2557.6
25/2/46	3 Ripe leaves	23.00	42.95	568.1
	3 Near ripe leaves	25.94	39.95	528.4
	7 Green leaves	21.29	37.57	496.9
	Laterals	20.75	6.12	80.9
	Stalks	16.69	86.83	1148.5
	Whole	20.01	213.42	2822.8
5/3/46	2 Ripe leaves	21.07	23.65	312.8
	3 Near ripe leaves	22.21	29.37	388.5
	5 Green leaves	22.31	36.03	476.6
	Laterals	11.21	11.54	152.6
	Stalks	15.75	73.28	969.3
	Whole	17.85	173.87	2299.8
12/3/46	3 Ripe leaves	20.25	32.71	432.7
	3 Near ripe leaves	21.38	25.63	339.0
	2 Green leaves	21.75	13.16	174.1
	Laterals	11.03	20.73	274.2
	Stalks	17.43	86.72	1147.0
	Whole	17.42	178.95	2367.0
26/3/46	5 Ripe leaves	20.49	33.72	446.0
	Laterals	13.51	71.87	950.6
	Stalks	22.88	121.11	1601.9
	Whole	18.49	226.70	2998.5

ripe leaves. In this season the yield of dry matter was higher than had been previously recorded at the Research Station and approximated the figure obtained on the silt loam in 1944-45. It is seen from Table XI that 19 leaves per plant were left after topping and that all of these were eventually harvested as ripe leaf, the last samples being obtained on 26th March, 113 days after the plants had been set in the field. The quantity of laterals at this date was unusually high, as was the figure for dry matter content of the stalks.

Yield data and percentages of dry matter in the plant separates for the 1946-47 season are set out in Table XII. At the beginning of this season growth appears to have been slower than in 1945-46, due probably to the long dry period from mid-November onwards. By mid-January the plants were 9-12 in. high, and by 6th February, 1947, they were beginning to send up flower stalks. Harvesting began on 11th February, the same date as in the previous season, but the total dry matter in the plants in 1947 was only 157 g. compared with 185 g. the year before. In spite of the dry weather the percentages of dry matter in the leaves at this date were lower than in the latter season by from 3 to 5 per cent. At topping 17 leaves were left on the plants, that is two less than in 1946. From mid-March onwards the leaves were distinctly yellowish green, whereas on the silt loam at this time the plants still looked dark green. By March the percentages of dry matter in corresponding separates were similar for the two seasons under discussion, but for the last sample on 27th March, 1947, the laterals and stalks showed lowered contents of dry matter, due possibly to the rains of 21st-24th March amounting to 2.18 in. The plants carried the maximum amount of leafage for the season at the sampling on 24th February, that is, at the same period in the season as in the previous year, but the maximum amount of dry matter in the whole plants at any one time was not reached until 10th March. Development of dry matter in the stalks appeared to cease after the middle of March. In 1947 only 15 of the 17 leaves left at topping were harvested, thus leaving two leaves with the laterals on the plants at the end of the season. Laterals at this time provided 10.8 g. of dry matter per plant or 191.5 lb. per acre, compared with the unusually high figure of 950.6 lb. at the end of the previous season.

(*b*) *Silt Loam Soil :*

Although the early part of the 1945-46 season was generally very dry, the silt loam area on which this experiment was set out was somewhat cold and damp, especially in the early part of the season. Consequently, as seen in Table XIII, the plants did not come away well after planting. Thus at the end of 44 days they contained approximately only half as much dry matter as the medium sand plants on the same date. The percentage of dry matter in the leaves was low but the stalks showed nearly the same figure as those on the lighter soil. On 25th February, 1946, when the first harvest was made, the plants having been topped to 13 leaves on the 20th, the whole plants yielded only 161.4 g. of dry matter per plant, as against 213.4 g. on this date at the Research Station ; moreover, the latter had already had 6 ripe leaves removed. On the silt loam the percentages of dry matter were relatively low in the leaves

TABLE XII. NUTRIENT INTAKE EXPERIMENT 1946-47 SEASON, TOBACCO RESEARCH STATION, MEDIUM SAND SOIL

Yield of Dry Matter and Percentage of Dry Matter

Date of Sampling.	Part of Plant	Dry Matter per cent	Dry Matter per plant. gm	Dry Matter per acre lb
3/12/46	Whole	9.89	0.56	7.4
15/1/47	Leaves	13.62	22.64	299.5
	Stalks	8.04	2.17	28.7
	Whole	12.84	24.81	328.2
29/1/47	Leaves	12.02	54.26	717.7
	Stalks	9.54	15.83	209.4
	Whole	11.35	70.09	927.1
6/2/47	Leaves	15.78	88.90	1175.9
	Stalks	8.44	31.63	418.4
	Whole	12.84	120.53	1594.3
11/2/47	3 Ripe leaves	12.31	16.63	220.0
	3 Near ripe leaves	14.65	25.03	331.1
	11 Green leaves	13.59	60.75	803.5
	Toppings	11.73	6.65	88.0
	Stalks	10.47	48.05	635.6
	Whole	12.92	157.11	2078.2
24/2/47	3 Ripe leaves	15.61	28.57	377.9
	3 Near ripe leaves	24.40	41.62	550.5
	8 Green leaves	20.24	51.12	676.2
	Stalks	13.13	62.76	830.1
	Whole	16.96	184.07	2434.7
10/3/47	3 Ripe leaves	19.77	37.81	500.1
	3 Near ripe leaves	21.72	35.19	465.5
	5 Green leaves	23.87	36.42	481.7
	Stalks	15.27	88.68	1173.0
	Whole	18.23	198.10	2620.3
19/3/47	3 Ripe leaves	21.18	32.41	428.7
	3 Near ripe leaves	21.18	22.69	300.1
	2 Green leaves	22.51	10.04	132.8
	Laterals	13.71	3.55	47.0
	Stalks	16.38	92.08	1217.9
	Whole	18.01	160.77	2126.5
27/3/47	3 Ripe leaves	20.02	26.34	348.4
	2 Near ripe leaves	25.18	14.48	191.5
	Laterals	12.04	10.80	142.8
	Stalks	15.69	91.95	1216.2
	Whole	16.60	143.57	1898.9

right throughout the season and especially at the end, following the rains of March and early April. The dry matter percentages for 9th April are low due to the plants not having dried completely after the heavy rain of 6.87 in. on 6th and 7th, followed by showers on 8th and 9th. As at the Research Station laterals constituted a large proportion of the dry matter of the plants at the end of the season. Production of dry matter on this area was much below that on the lighter soil in this season. Harvested leaf corresponded to 103.3 g. per plant as against 179.1 g. at the Research Station; these figures represent yields of 1,366.5 lb. and 2,369.3 lb. per acre.

Table XIII. Nutrient Intake Experiment 1945-46 Season, Light-phase Silt-loam Soil
Yield of Dry Matter and Percentage of Dry Matter

Date of Sampling.	Part of Plant	Dry Matter per cent	Dry Matter per plant gm.	Dry Matter per acre lb
3/12/45		24.32	0.98	13.0
16/1/46	Leaves	13.66	17.19	227.4
	Stalks	8.30	1.52	20.1
	Whole	12.98	18.71	247.5
30/1/46	Leaves	16.97	53.81	711.7
	Stalks	9.25	10.12	133.9
	Whole	14.99	63.93	845.6
11/2/46	Leaves	14.78	87.17	1153.0
	Stalks	13.71	41.91	554.3
	Whole	14.41	129.08	1707.3
25/2/46	3 Ripe leaves	19.45	25.95	343.2
	3 Near ripe leaves	18.25	24.76	327.5
	7 Green leaves	22.00	46.81	619.2
	Toppings (20/2/46)		4.96	65.6
	Laterals	13.74	2.02	26.7
	Stalks	14.59	56.92	752.9
	Whole		161.42	2135.1
12/3/46	3 Ripe leaves	17.27	25.90	342.6
	3 Near ripe leaves	17.05	20.54	271.7
	4 Green leaves	17.66	16.25	214.9
	Laterals	10.98	1.01	13.4
	Stalks	16.20	62.50	826.7
	Whole	16.66	126.20	1669.3
26/3/46	3 Ripe leaves	15.19	25.78	341.0
	4 Near ripe leaves	15.07	20.09	265.7
	Laterals	9.60	33.55	443.8
	Stalks	15.00	61.11	808.3
	Whole	13.26	140.53	1858.8
9/4/46	4 Leaves	16.12	25.68	339.7
	Laterals	12.11	88.79	1174.4
	Stalks	17.24	83.01	1098.0
	Whole	14.38	197.48	2612.1

The month before planting and the month immediately after planting in the 1946-47 season were unusually dry, and the plants, set out on the same small field that was used for the experiment on the silt loam in the previous season, did not come away at all well. As will be seen by comparison of the yield data in Table XIV with those for corresponding dates in Table XIII, the development of dry matter was very slow, although by the time the first harvest was made on 10th March, 1947, the plants were larger than were those of the previous year on 25th February, 1946, when the first harvest of the 1945-46 season was taken. It will be noticed too that the plants were topped very high, 21 leaves being left on the plants. Percentages of dry matter were low during the earlier part of the season in 1947 but by the end of the season in April the percentages of dry matter were generally similar to corresponding separates at the end of the previous season. The plants carried the maximum amount of dry matter for the season at the sampling of 10th March, 1947, with a total of 183.3 g., corresponding to 2,424.0 lb. per acre. Yield of harvested leaves was 117.6 g. per plant, or 1,555.0 lb. per acre, these figures being approximately 14 per cent. higher than those of the previous season. It is of interest, too, to note that the stalks of the plants were appreciably larger in 1946-47.

TABLE XIV. NUTRIENT INTAKE EXPERIMENT 1946-47 SEASON, LIGHT-PHASE SILT-LOAM SOIL
Yield of Dry Matter and Percentage of Dry Matter

Date of Sampling.	Part of Plant.	Dry Matter per cent.	Dry Matter per plant. gm.	Dry Matter per acre. lb.
3/12/46	Whole	9.89	0.56	7.4
29/1/47	Leaves	13.20	16.06	212.4
	Stalks	11.24	1.91	25.3
	Whole	12.96	17.97	237.7
11/2/47	Leaves	15.34	46.79	618.9
	Stalks	9.16	8.95	118.4
	Whole	13.84	55.74	737.3
24/2/47	Leaves	14.12	77.23	1021.5
	Stalks	8.47	20.83	275.5
	Whole	12.36	98.06	1297.0
10/3/47	3-4 Ripe leaves lugs	13.92	15.68	207.4
	3 Near ripe leaves	15.77	22.23	294.0
	15 Green leaves	17.52	78.90	1043.6
	Stalks	12.43	56.19	743.2
	Toppings	13.77	10.27	135.8
	Whole	14.91	183.27	2424.0
27/3/47	3 Ripe leaves	14.23	23.23	307.3
	3 Near ripe leaves	15.79	24.43	323.1
	12 Green leaves	17.98	63.81	844.0
	Laterals	11.05	2.32	30.7
	Stalks	14.28	66.62	881.2
	Whole	15.55	180.41	2386.3
2/4/47	3 Ripe leaves	15.17	25.56	338.1
	3 Near ripe leaves	15.05	20.62	272.7
	9 Green leaves	15.92	38.07	503.5
	Stalks	14.30	79.51	1051.7
	Whole	14.88	163.76	2166.0
9/4/47	3 Ripe leaves	17.34	28.71	379.7
	3 Near ripe leaves	16.26	25.15	332.7
	6 Green leaves	17.32	31.50	416.7
	Laterals	12.01	2.27	30.0
	Stalks	15.44	85.05	1125.0
	Whole	16.11	172.68	2284.1
16/4/47	3 Ripe leaves	16.64	24.38	322.5
	3 Near ripe leaves	16.52	19.16	253.4
	3 Green leaves	17.00	11.71	154.9
	Laterals	10.96	4.78	632.2
	Stalks	16.60	107.04	1415.8
	Whole	16.38	167.07	2209.8

TABLE XV. CHEMICAL COMPOSITION OF TOBACCO, NUTRIENT INTAKE EXPERIMENT, TOBACCO RESEARCH STATION 1945-46 SEASON

Expressed as percentage on sand-free dry matter basis

Date of Sampling	Part of Plant.	Insoluble Ash.	Soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N.
3/12/45	Whole	0.35	9.99	1.76	0.39	0.62	4.06	1.43
16/1/46	Leaves	1.23	16.05	3.33	0.81	0.72	4.96	3.16
	Stalks	0.53	14.90	1.52	0.55	0.77	6.75	2.41
23/1/46	Leaves	0.17	15.98	3.71	0.84	0.77	4.72	3.34
	Stalks	0.11	14.79	1.70	0.51	0.68	6.66	2.17
30/1/46	Leaves	0.34	12.66	3.05	0.66	0.54	4.01	2.57
	Stalks	0.70	11.61	1.28	0.40	0.50	5.42	1.65
6/2/46	Leaves	0.57	11.63	2.85	0.58	0.48	3.73	2.28
	Stalks	0.11	10.32	1.02	0.35	0.49	4.48	1.36
11/2/46	3 Ripe leaves	0.19	14.84	4.72	0.88	0.35	3.16	1.32
	3 Near ripe leaves	0.17	10.68	2.71	0.56	0.35	3.05	1.75
	Green leaves	0.13	9.11	1.79	0.48	0.44	3.31	2.35
	Stalks and flower heads	0.16	9.57	1.08	0.36	0.44	4.28	1.43
18/2/46	3 Ripe leaves	0.30	13.79	4.35	0.91	0.38	3.04	1.66
	3 Near ripe leaves	0.43	10.72	2.10	0.59	0.42	3.07	2.00
	10 Green leaves	0.17	9.05	2.77	0.57	0.46	2.98	2.41
	Toppings (13/2/46)	0.53	10.84	1.07	0.63	0.99	4.43	4.04
	Stalks	0.13	8.14	0.98	0.35	0.38	3.64	1.35
25/2/46	3 Ripe leaves	0.15	10.62	3.73	0.63	0.45	2.74	1.73
	3 Near ripe leaves	0.28	9.89	2.75	0.65	0.43	3.05	1.87
	7 Green leaves	0.04	8.88	2.20	0.59	0.51	2.74	2.40
	Laterals	0.60	12.92	1.41	0.68	1.41	5.75	4.82
	Stalks	0.08	8.96	1.05	0.41	0.45	3.98	1.48
5/3/46	3 Ripe leaves	0.51	10.41	2.83	0.65	0.44	2.54	1.63
	3 Near ripe leaves	0.35	8.79	2.34	0.60	0.56	2.68	1.86
	5 Green leaves	0.38	8.94	2.34	0.64	0.44	2.52	2.12
	Laterals	0.89	12.78	1.50	0.84	1.14	5.81	3.74
	Stalks	0.20	7.75	1.09	0.35	0.36	3.46	1.12
12/3/46	3 Ripe leaves	0.40	9.21	2.61	0.61	0.45	2.35	1.61
	3 Near ripe leaves	0.15	9.06	2.54	0.65	0.47	2.29	1.92
	2 Green leaves	0.14	9.34	2.58	0.69	0.46	2.31	2.12
	Laterals	0.02	13.51	1.70	0.74	1.05	5.60	3.45
	Stalks	0.18	8.54	1.19	0.37	0.38	3.58	0.99
26/3/46	5 Ripe leaves	0.15	11.05	3.35	0.84	0.61	2.47	2.00
	Laterals	0.06	11.19	1.75	0.62	0.74	4.59	2.46
	Stalks	0.01	6.78	0.92	0.21	0.32	2.88	0.87

Chemical Composition of Plant Separates :

Chemical data for plant separates from the Tobacco Research Station are set out in Tables XV and XVI, the former referring to the 1945-46 season and the latter to the 1946-47 season. Seedlings when set in the field were especially rich in potash, but not in respect of the other constituents determined when comparison is made with later samples. In 1946-47 the seedlings were appreciably richer in all constituents than in 1945-46. As the plants developed the leaves became relatively rich in soluble ash, lime, magnesia, phosphoric acid, potash and nitrogen until about the end of January when all commenced to fall in concentration. When harvesting began on 11th February, in each season the ripe leaves were well supplied with soluble ash, lime and magnesia but were lower in

TABLE XVI. CHEMICAL COMPOSITION OF TOBACCO, NUTRIENT INTAKE EXPERIMENT, TOBACCO RESEARCH STATION 1946-47 SEASON
Expressed as percentage on sand-free dry matter basis

Date of Sampling.	Part of Plant	Insoluble Ash	Soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N
3/12/46	Whole	0.90	14.13	2.75	0.59	0.75	4.76	2.49
15/1/47	Leaves	0.34	15.72	3.24	0.65	0.61	5.17	2.18
	Stalks	0.42	15.80	1.54	0.50	0.75	7.40	2.30
29/1/47	Leaves	0.26	17.63	4.02	0.95	0.75	5.54	3.55
	Stalks	0.10	12.53	1.18	0.43	0.67	6.00	2.46
6/2/47	Leaves	0.36	15.04	3.45	0.71	0.60	4.40	2.82
	Stalks	0.10	12.07	1.22	0.41	0.69	5.60	2.07
11/2/47	3 Ripe leaves	0.43	20.70	6.71	1.33	0.41	3.58	1.78
	3 Near ripe leaves	0.16	14.45	4.13	0.84	0.47	4.28	2.32
	11 Green leaves	0.20	10.79	2.36	0.57	0.61	4.82	2.98
	Toppings	0.42	10.57	0.95	0.64	1.38	4.51	4.50
	Stalks	0.19	10.03	1.06	0.35	0.52	4.67	1.67
24/2/47	3 Ripe leaves	0.41	13.08	3.78	0.78	0.45	3.32	1.59
	3 Near ripe leaves	0.36	9.58	2.40	0.53	0.41	3.13	1.60
	8 Green leaves	0.26	8.51	1.75	0.49	0.50	3.18	2.23
	Stalks	0.13	9.46	1.22	0.40	0.47	4.06	1.43
10/3/47	3 Ripe leaves	0.48	9.34	2.57	0.56	0.48	2.92	1.17
	3 Near ripe leaves	0.23	8.28	1.94	0.46	0.46	2.77	1.37
	5 Green leaves	0.29	7.87	1.79	0.51	0.44	2.69	1.67
	Stalks	0.09	8.27	1.12	0.37	0.48	3.68	1.25
19/3/47	3 Ripe leaves	0.29	8.99	2.31	0.59	0.53	2.70	1.56
	3 Near ripe leaves	0.22	8.51	2.09	0.61	0.47	2.60	1.84
	2 Green leaves	0.23	8.18	2.08	0.61	0.50	2.37	2.16
	Laterals	1.23	11.53	1.27	0.71	1.04	4.61	3.70
	Stalks	0.02	8.29	1.19	0.42	0.45	3.62	1.25
27/3/47	3 Ripe leaves	0.08	9.86	2.75	0.74	0.59	2.40	2.00
	2 Near ripe leaves	0.10	9.90	2.82	0.76	0.62	2.35	2.30
	Laterals	0.54	14.85	1.53	0.89	1.12	5.22	3.74
	Stalks	0.04	8.52	1.23	0.40	0.41	3.34	1.28

potash and nitrogen than the remaining leaves. There was a tendency from then onwards for soluble ash, lime, potash and nitrogen to decrease in the leaves, while magnesia and phosphoric acid moved towards relatively constant values until the end of the season, when they increased in company with most of the other constituents.

Taking now the changes in leaf composition due to maturation, that is, in passing from the " nearly ripe " to the " ripe " condition, it is seen that in both seasons potash and nitrogen decreased as the leaf ripened, soluble ash and lime increased, while in general magnesia and phosphoric acid remained fairly constant.

Changes in chemical composition of the stalks are noticeable. Soluble ash and potash, and to some extent nitrogen contents decrease steadily throughout the season, but lime, magnesia and phosphoric acid contents by early February have fallen to values which remain fairly constant throughout the remainder of the season.

The plants of the 1946-47 season began with a greater concentration of nutrients in their dry matter than those of the 1945-46 season. This advantage was maintained by the leaves in general only until the end of February or early in March ; from then onwards they showed lower values than the corresponding separates of the 1945-46 season. On the other hand the stalks were generally richer in all constituents in 1946-47 at corresponding periods throughout the season. Young growth (laterals) was very rich, in comparison with older leaves on the plants, in all constituents determined, with the exception of lime. Potash and nitrogen contents were particularly high.

In Tables XVII and XVIII are set out the chemical data obtained on material from the 1945-46 and 1946-47 seasons' experiments on the silt loam soil. The variations in composition follow in general those shown by the Research Station data. It is notable however that soluble ash, lime, magnesia, potash and nitrogen contents were often appreciably higher in the samples from the silt loam than in those from the medium sand of the Research Station. On the other hand phosphoric acid was lower on the heavier soil. This was particularly marked in the 1946-47 season (Table XVIII). The ripe leaves on the silt loam area were distinguished by their high soluble ash and lime contents, these being at maximum values in the bottom leaves, the former rising to 24.51 per cent. and the latter constituent to 7.49 per cent. CaO. In the previous season the figures were 15.02 per cent. and 4.76 per cent. respectively. Potash was high in these leaves, being 5.76 per cent. K_2O. Young laterals harvested on 27th March, 1947, showed 4.97 per cent. K_2O and 6.01 per cent. N, on the dry basis ; even at the end of the season the much heavier growth on 16th April gave 5.55 per cent. and 5.11 per cent. respectively for these constituents. These figures were much higher than were obtained for laterals in the 1945-46 season. While potash in the corresponding material from the Research Station in 1946-47 approached these values the percentage of nitrogen was much lower. Stalks of plants grown on the heavier soil were richer in nutrients in both seasons than were those on the medium sand, this difference being particularly marked in respect of the nitrogen contents.

TABLE XVII. CHEMICAL COMPOSITION OF TOBACCO, NUTRIENT INTAKE EXPERIMENT, LIGHT-PHASE SILT-LOAM SOIL, 1945-46 SEASON

Expressed as percentage on sand-free dry matter basis

Date of Sampling	Part of Plant.	Insoluble Ash.	Soluble Ash.	Lime CaO	Magnesia MgO.	Phosphoric Acid P_2O_5.	Potash K_2O.	Total Nitrogen N.
3/12/45	Whole	0.35	9.99	1.76	0.39	0.62	4.06	1.43
16/1/46	Leaves	0.39	16.25	3.36	0.85	0.61	5.42	3.91
	Stalks	0.38	15.60	1.38	0.54	0.74	7.11	3.35
30/1/46	Leaves	0.14	13.59	3.30	0.76	0.42	4.54	3.20
	Stalks	0.09	13.16	1.48	0.50	0.45	6.06	2.21
11/2/46	Leaves	0.09	13.52	3.59	0.87	0.40	3.61	2.79
	Stalks and flower heads	0.29	10.54	1.16	0.39	0.33	4.46	1.66
25/2/46	3 Ripe leaves	0.43	15.02	4.76	1.06	0.38	3.07	1.93
	3 Near ripe leaves	0.16	12.19	3.44	0.91	0.38	3.08	2.58
	7 Green leaves	0.22	9.83	2.62	0.78	0.43	2.55	3.00
	Toppings (20/2/46)	0.45	11.77	1.06	0.63	1.22	4.57	4.45
	Laterals	0.40	11.84	1.62	0.74	1.44	4.66	2.77
	Stalks	0.22	8.44	1.09	0.40	0.41	3.75	1.31
12/3/46	3 Ripe leaves	0.01	13.78	4.01	1.08	0.52	2.77	3.18
	3 Near ripe leaves	0.06	12.29	3.87	1.09	0.54	2.63	3.61
	4 Green leaves	0.03	12.85	3.49	1.05	0.51	2.76	4.39
	Laterals	0.79	15.28	2.53	0.88	1.27	5.15	5.04
	Stalks	0.10	10.51	1.43	0.50	0.38	3.82	1.61
26/3/46	3 Ripe leaves	0.18	16.29	5.17	1.27	0.50	3.20	3.26
	4 Near ripe leaves	0.16	15.66	4.79	1.30	0.54	3.06	3.56
	Laterals	0.32	14.79	2.43	0.89	0.99	5.41	4.36
	Stalks	0.22	8.88	1.36	0.46	0.31	3.64	1.56
9/4/46	4 Leaves	0.21	16.10	5.30	1.33	0.56	2.47	3.12
	Laterals	0.19	12.58	2.12	0.72	0.80	3.96	3.31
	Stalks	0.14	7.75	1.19	0.36	0.30	3.23	1.28

TABLE XVIII. CHEMICAL COMPOSITION OF TOBACCO, NUTRIENT INTAKE EXPERIMENT, LIGHT-PHASE SILT-LOAM SOIL, 1946-47 SEASON

Expressed as percentage on sand-free dry matter basis

Date of Sampling.	Part of Plant.	In-soluble Ash	Soluble Ash.	Lime CaO.	Magnesia MgO.	Phosphoric Acid P_2O_5	Potash K_2O.	Total Nitrogen N.
3/12/46	Whole	0.90	14.13	2.75	0.59	0.75	4.76	2.49
29/1/47	Leaves	0.29	18.70	4.13	0.98	0.54	6.25	4.23
	Stalks	0.14	14.08	1.50	0.43	-	6.45	3.01
11/2/47	Leaves	0 30	16.42	3.95	0.97	0.41	5.11	3.58
	Stalks	0.15	14.07	1.36	0.51	0.45	6.41	2.92
24/2/47	Leaves	0.18	17.02	4.09	1.00	0.45	5.27	3.61
	Stalks	0.25	13.20	1.31	0.50	0.38	6.59	2.90
10/3/47	3-4 Ripe leaves lugs	0.25	24.51	7.49	1.65	0.25	5.76	2.09
	3 Near ripe leaves	0.25	17.09	4.98	1.19	0.30	4.39	2.77
	15 Green leaves	0.11	11.64	2.80	0.78	0.40	3.78	3.47
	Stalks	0.13	9.57	1.01	0.39	0.32	4.20	1.98
	Toppings	0.27	9.78	1.04	0.58	1.11	4.26	4.38
27/3/47	3 Ripe leaves	0.56	17.10	5.38	1.33	0.28	3.85	2.23
	3 Near ripe leaves	0.43	13.15	3.67	0.99	0.31	3.47	2.58
	12 Green leaves	0.31	10.62	2.69	0.83	0.38	3.11	3.24
	Laterals	0.11	13.10	1.44	0.75	1.67	4.97	6.01
	Stalks	0.05	8.55	1.08	0.43	0.27	3.46	1.61
2/4/47	3 Ripe leaves	0.50	15.54	4.59	1.19	0.43	3.55	2.78
	3 Near ripe leaves	0.53	13.75	3.80	1.02	0.47	3.64	3 13
	9 Green leaves	0.57	12.72	3.37	0.98	0.58	3.20	3.70
	Stalks	0.06	9.66	1.51	0.45	0.29	3.59	3.63
9/4/47	3 Ripe leaves	0.84	14.88	4.71	1.21	0.51	3.13	3.05
	2 Near ripe leaves	0.46	13.19	3.85	1.05	0.54	3.03	2.18
	6 Green leaves	0.37	12.10	3.34	0.97	0.59	2.75	3.69
	Laterals	0.31	13.17	1.45	0.75	1.24	5.27	4.77
	Stalks	0.03	8.93	1.23	0.44	0.42	3.36	1.79
16/4/47	3 Ripe leaves	0.79	12.72	3.92	1.14	0.58	2.78	4.63
	3 Near ripe leaves	0.46	13.67	3.82	1.19	0.60	2.78	3.70
	3 Green leaves	0.74	13.68	4.16	1.28	0.67	2.94	4.18
	Laterals	0.40	13.17	1.60	0.75	1.26	5.55	5.11
	Stalks	0.00	8.61	1.33	0.50	0.36	3.19	1.69

TABLE XIX. INTAKE OF NUTRIENTS ON PER PLANT BASIS, 1945 46 SEASON, TOBACCO RESEARCH STATION (MEDIUM SAND SOIL)
Expressed in grams on sand-free dry matter basis

Date of Sampling.	Part of Plant.	Insoluble Ash	Soluble Ash.	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N
3/12/45	Whole	0.003	0.10	0.02	0.004	0.006	0.04	0.014
16/1/46	Leaves	0.37	4.78	0.99	0.24	0.21	1.48	0.94
	Stalks	0.02	0.67	0.07	0.02	0.03	0.31	0.11
	Whole	0.39	5.45	1.06	0.26	0.24	1.79	1.05
23/1/46	Leaves	0.09	8.40	1.95	0.44	0.37	2.48	1.76
	Stalks	0.01	1.64	0.19	0.06	0.08	0.74	0.24
	Whole	0.10	10.04	2.14	0.50	0.45	3.22	2.00
30/1/46	Leaves	0.27	10.29	2.48	0.54	0.44	3.26	2.09
	Stalks	0.16	2.61	0.29	0.09	0.11	1.22	0.37
	Whole	0.43	12.90	2.77	0.63	0.55	4.48	2.46
6/2/46	Leaves	0.53	10.78	2.64	0.54	0.45	3.46	2.11
	Stalks	0.05	4.79	0.47	0.16	0.23	2.07	0.63
	Whole	0.58	15.57	3.11	0.70	0.68	5.53	2.74
11/2/46	3 Ripe leaves	0.04	3.05	0.97	0.18	0.07	0.65	0.27
	3 Near ripe leaves	0.05	3.18	0.81	1.67	1.04	0.91	0.52
	Green leaves	0.09	5.97	1.17	0.31	0.29	2.17	1.54
	Stalks and flower heads	0.11	6.66	0.75	0.25	0.31	2.98	1.00
	Whole	0.29	18.86	3.70	2.41	1.71	6.71	3.33
18/2/46	3 Ripe leaves	0.08	3.53	1.11	0.23	0.10	0.78	0.42
	3 Near ripe leaves	0.14	3.47	0.68	0.19	0.14	0.99	0.65
	10 Green leaves	0.10	5.17	1.58	0.33	0.26	1.70	1.38
	Toppings (13/2/46)	0.06	1.15	0.11	0.07	0.10	0.47	0.43
	Stalks	0.09	5.51	0.66	0.24	0.26	2.47	0.91
	Whole	0.47	18.83	4.14	1.06	0.86	6.41	3.79
25/2/46	3 Ripe leaves	0.06	4.56	1.60	0.27	0.19	1.18	0.74
	3 Near ripe leaves	0.11	3.95	1.10	0.26	0.17	1.22	0.75
	7 Green leaves	0.01	3.34	0.83	0.22	0.19	1.03	0.90
	Laterals	0.04	0.79	0.09	0.04	0.09	0.35	0.29
	Stalks	0.07	7.78	0.91	0.36	0.39	3.46	1.29
	Whole	0.29	20.42	4.53	1.15	1.03	7.24	3.97
5/3/46	2 Ripe leaves	0.12	2.46	0.67	0.15	0.10	0.60	0.39
	3 Near ripe leaves	0.10	2.58	0.69	0.18	0.14	0.79	0.55
	2 Green leaves	0.14	3.22	0.84	0.23	0.16	0.91	0.76
	Laterals	0.10	1.47	0.17	0.10	0.13	0.67	0.43
	Stalks	0.15	5.68	0.80	0.26	0.26	2.54	0.82
	Whole	0.61	15.41	3.17	0.92	0.79	5.51	2.95
12/3/46	3 Ripe leaves	0.13	3.01	0.85	0.20	0.15	0.77	0.53
	3 Near ripe leaves	0.04	2.32	0.65	0.17	0.12	0.59	0.49
	2 Green leaves	0.02	1.23	0.34	0.09	0.06	0.30	0.28
	Laterals	0.00	2.80	0.35	0.15	0.22	1.16	0.72
	Stalks	0.16	7.41	1.03	0.32	0.33	3.10	0.86
	Whole	0.35	16.77	3.22	0.93	0.88	5.92	2.88
26/3/46	5 Ripe leaves	0.05	3.73	1.13	0.28	0.21	0.83	0.67
	Laterals	0.04	8.04	1.26	0.45	0.53	3.30	1.77
	Stalks	0.01	8.21	1.11	0.25	0.39	3.49	1.05
	Whole	0.10	19.98	3.50	0.98	1.13	7.62	3.49

TABLE XX INTAKE OF NUTRIENTS ON PER PLANT BASIS, 1946-47 SEASON, TOBACCO RESEARCH STATION (MEDIUM SAND SOIL)
Expressed in grams on sand-free dry matter basis

Date of Sampling	Part of Plant	In-soluble Ash	Soluble Ash.	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N
3/12/46	Whole	0.005	0.08	0.02	0.003	0.004	0.03	0.01
15/1/47	Leaves	0.08	3.56	0.73	0.15	0.14	1.17	0.49
	Stalks	0.01	0.34	0.03	0.01	0.02	0.16	0.05
	Whole	0.09	3.90	0.76	0.16	0.16	1.33	0.54
29/1/47	Leaves	0.14	9.57	2.18	0.52	0.41	3.01	1.93
	Stalks	0.02	1.98	0.19	0.07	0.11	0.95	0.39
	Whole	0.16	11.55	2.37	0.59	0.52	3.96	2.32
6/2/47	Leaves	0.32	13.37	3.42	0.63	0.53	3.91	2.51
	Stalks	0.03	3.82	0.39	0.13	0.21	1.77	0.65
	Whole	0.35	17.19	3.81	0.76	0.74	5.68	3.16
11/2/47	3 Ripe leaves	0.07	3.44	1.12	0.22	0.07	0.60	0.30
	3 Near ripe leaves	0.04	3.62	1.03	0.20	0.12	1.07	0.58
	11 Green leaves	0.12	6.55	1.43	0.35	0.37	2.93	1.81
	Toppings	0.03	0.70	0.06	0.04	0.09	0.30	0.30
	Stalks	0.09	4.82	0.51	0.16	0.25	2.24	0.80
	Whole	0.35	19.13	4.15	0.97	0.90	7.14	3.79
24/2/47	3 Ripe leaves	0.12	3.74	1.08	0.22	0.13	0.95	0.45
	3 Near ripe leaves	0.15	3.98	1.00	0.22	0.17	1.30	0.67
	8 Green leaves	0.13	4.35	0.90	0.25	0.26	1.63	1.14
	Stalks	0.08	5.94	0.76	0.25	0.29	2.55	0.90
	Whole	0.48	18.01	3.74	0.94	0.85	6.43	3.16
10/3/47	3 Ripe leaves	0.18	3.53	0.97	0.21	0.18	1.10	0.44
	3 Near ripe leaves	0.08	2.91	0.68	0.16	0.16	0.97	0.48
	5 Green leaves	0.11	2.87	0.65	0.19	0.16	0.97	0.61
	Stalks	0.08	7.33	1.00	0.33	0.43	3.26	1.11
	Whole	0.45	16.64	3.30	0.89	0.93	6.30	2.64
19/3/47	3 Ripe leaves	0.09	2.91	0.74	0.19	0.17	0.88	0.51
	3 Near ripe leaves	0.05	1.93	0.47	0.14	0.11	0.59	0.42
	2 Green leaves	0.02	0.82	0.21	0.06	0.05	0.24	0.22
	Laterals	0.04	0.41	0.05	0.03	0.04	0.16	0.13
	Stalks	0.02	7.63	1.10	0.39	0.42	3.33	1.15
	Whole	0.22	13.70	2.57	0.81	0.79	5.20	2.43
27/3/47	3 Ripe leaves	0.02	2.60	0.72	0.20	0.16	0.63	0.53
	2 Near ripe leaves	0.01	1.43	0.41	0.11	0.09	0.34	0.33
	Laterals	0.06	1.60	0.17	0.10	0.12	0.56	0.40
	Stalks	0.04	7.83	1.13	0.37	0.39	3.07	1.18
	Whole	0.13	13.46	2.43	0.78	0.76	4.60	2.44

Intake of Nutrients :

Calculations on a similar basis to those reported above for the 1944-45 experiments have been made using the data of the 1945-46 and 1946-47 experiments. For the medium sand area the results are given in Table XIX for the 1945-46 season, and in Table XX for the 1946-47 season.

In keeping with the slightly better growth in the 1945-46 season the intake of nutrients was greater in the very early part of the season than in the 1946-47 season, but at the time of the first harvest more soluble ash, lime, potash and nitrogen had been absorbed in the latter season. Absorption of phosphoric acid and magnesia was appreciably greater at this stage in 1945-46. At later periods plants of the 1945-46 season contained a greater weight of nutrients at a given period than those of the next season. Nutrient residues in the plants when harvesting was completed were appreciably greater, especially for potash and nitrogen, in 1945-46; these latter constituents provided an excess of 2.8 g. of K_2O and 0.9 g. of N per plant in favour of the 1945-46 crop.

TABLE XXI. INTAKE OF NUTRIENTS ON PER PLANT BASIS, 1945-46 SEASON, LIGHT-PHASE SILT-LOAM SOIL

Expressed in grams on sand-free dry matter basis

Date of Sampling.	Part of Plant.	Insoluble Ash.	Soluble Ash.	Lime CaO.	Magnesia MgO.	Phosphoric Acid P_2O_5.	Potash K_2O.	Total Nitrogen N.
3/12/45	Whole	0.003	0.10	0.02	0.004	0.006	0.04	0.014
16/1/46	Leaves	0.07	2.79	0.58	0.15	0.10	0.93	0.67
	Stalks	0.006	0.24	0.02	0.01	0.01	0.11	0.05
	Whole	0.076	3.03	0.60	0.16	0.11	1.04	0.72
30/1/46	Leaves	0.08	7.31	1.78	0.41	0.23	2.44	1.72
	Stalks	0.01	1.33	0.15	0.05	0.05	0.61	0.22
	Whole	0.09	8.64	1.93	0.46	0.28	3.05	1.94
11/2/46	Leaves	0.08	11.79	3.13	0.76	0.35	3.15	2.43
	Stalks and flower heads	0.12	4.42	0.49	0.16	0.14	1.87	0.70
	Whole	0.20	16.21	3.62	0.92	0.49	5.02	3.13
25/2/46	3 Ripe leaves	0.11	3.90	1.24	0.28	0.10	0.80	0.56
	3 Near ripe leaves	0.04	3.02	0.85	0.23	0.10	0.76	0.64
	7 Green leaves	0.10	4.60	1.23	0.37	0.20	1.19	1.40
	Toppings (20/2/46)	0.02	0.58	0.05	0.03	0.06	0.23	0.22
	Laterals	0.01	0.24	0.03	0.01	0.03	0.09	0.06
	Stalks	0.13	4.80	0.62	0.23	0.23	2.13	0.75
	Whole	0.41	17.14	4.02	1.15	0.72	5.20	3.57
12/3/46	3 Ripe leaves	0.00	3.57	1.04	0.28	0.13	0.72	0.82
	3 Near ripe leaves	0.01	2.52	0.79	0.22	0.11	0.54	0.74
	4 Green leaves	0.005	2.08	0.57	0.17	0.08	0.44	0.71
	Laterals	0.01	0.15	0.03	0.01	0.01	0.05	0.05
	Stalks	0.06	6.57	0.89	0.31	0.24	2.39	1.01
	Whole	0.085	14.89	3.32	0.99	0.57	4.14	3.33
26/3/46	3 Ripe leaves	0.05	4.20	1.33	0.33	0.13	0.82	0.84
	4 Near ripe leaves	0.03	3.15	0.96	0.26	0.11	0.61	0.72
	Laterals	0.11	4.96	0.82	0.30	0.33	1.82	1.46
	Stalks	0.13	5.43	0.83	0.28	0.19	2.22	0.95
	Whole	0.32	17.74	3.94	1.17	0.76	5.47	3.97
9/4/46	4 Leaves	0.05	4.13	1.36	0.34	0.14	0.63	0.80
	Laterals	0.17	11.17	1.88	0.64	0.71	3.52	2.94
	Stalks	0.12	6.43	0.99	0.30	0.25	2.68	1.06
	Whole	0.34	21.73	4.23	1.28	1.10	6.83	4.80

TABLE XXII. INTAKE OF NUTRIENTS ON PER PLANT BASIS, 1946-47 SEASON, LIGHT-PHASE SILT-LOAM SOIL

Expressed in grams on sand-free dry matter basis

Date of Sampling.	Part of Plant.	Insoluble Ash.	Soluble Ash	Lime CaO	Magnesia MgO.	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N
3/12/46	Whole	0.005	0.08	0 02	0.003	0.004	0 03	0.014
29/1/47	Leaves	0.04	3 00	0.66	0.16	0.09	1.00	0.68
	Stalks	0.003	0.27	0.03	0.01		0.12	0.06
	Whole	0.043	3.27	0.69	0.17	0.09	1 12	0.74
11/2/47	Leaves	0.14	7.68	1.85	0.46	0.19	2.39	1.68
	Stalks	0.01	1.26	0.12	0.05	0.04	0 57	0.26
	Whole	0.15	8.94	1.97	0 51	0.23	2.96	1.94
24/2/47	Leaves	0.14	13.14	3.16	0.78	0.35	4.07	2.79
	Stalks	0.05	2.75	0.27	0.10	0.08	1.37	0.60
	Whole	0.19	15.89	3.43	0.88	0.43	5.44	3.39
10/3/47	3-4 Ripe leaves lugs	0.04	3.84	1.17	0.26	0.04	0.90	0.33
	3 Near ripe leaves	0.05	3.80	1.11	0.26	0 07	0 98	0.62
	15 Green leaves	0.09	9.18	2.21	0.62	0.32	2.98	2.74
	Stalks	0.07	5.38	0.57	0.21	0 18	2.36	1.11
	Toppings	0.03	1.00	0.11	0.06	0.11	0.44	0.45
	Whole	0.28	23.20	5.17	1.41	0.72	7.66	5.25
27/3/47	3 Ripe leaves	0.13	3.97	1.25	0.31	0.07	0.89	0.52
	3 Near ripe leaves	0.11	3.21	0.90	0.24	0.08	0.85	0.63
	12 Green leaves	0.20	6.78	1.71	0.52	0.24	1.98	2.07
	Laterals	0.003	0.30	0.03	0.02	0.04	0.12	0.14
	Stalks	0.03	5.70	0.72	0.29	0.18	2.31	1.07
	Whole	0.47	19.96	4.61	1.38	0.61	6.15	4.43
2/4/47	3 Ripe leaves	0.13	3.97	1.17	0.30	0.11	0.91	0.71
	3 Near ripe leaves	0.11	2.84	0.78	0.21	0.10	0.75	0.65
	9 Green leaves	0.22	4.84	1.28	0.37	0.22	1.22	1.41
	Stalks	0.05	7.68	1.20	0.36	0.23	2.85	2.89
	Whole	0.51	19.33	4.43	1.24	0.66	5.73	5.66
9/4/47	3 Ripe leaves	0.24	4.27	1.35	0.35	0.15	0.90	0.88
	3 Near ripe leaves	0.12	3.32	0.97	0.26	0.14	0.76	0.55
	6 Green leaves	0.12	3.81	1.05	0.31	0.19	0.87	1.16
	Laterals	0.01	0.30	0.03	0.02	0.03	0.12	0.11
	Stalks	0.03	7.59	1.05	0.37	0.36	2.86	1.52
	Whole	0.52	19.29	4.45	1.31	0.87	5.51	4.22
16/4/47	3 Ripe leaves	0.19	3.10	0.96	0.28	0.14	0.68	1.13
	3 Near ripe leaves	0.09	2.62	0.73	0.23	0.11	0.53	0.71
	3 Green leaves	0.09	1.60	0.49	0.15	0.08	0.34	0.49
	Laterals	0.02	0.63	0.08	0.04	0.06	0.27	0.24
	Stalks	0.00	9.22	1.43	0.54	0.39	3.41	1.81
	Whole	0.39	17.17	3.69	1.24	0.78	5.23	4.38

Data on a precisely similar basis to that given above for the plants grown at the Research Station have been obtained for the crops on the silt loam area ; these are set out in Tables XXI and XXII. By the time of the first harvest on 25th February, 1946, minerals corresponding to 17.14 g. per plant had been absorbed; of these lime constituted 4.02 g., magnesia 1.15 g., phosphoric acid 0.72 g., potash 5.20 g., K_2O and nitrogen 3.57 g. In the following season, by 24th February somewhat less of all constituents except potash had been taken up by the plant. At the time of the first harvest on 10th March, 1947, larger quantities of all nutrients except phosphoric acid had been absorbed than at the time of the first harvest in 1946. It is interesting to note that the nutrients removed in each harvest of ripe leaves tends to be constant, except for nitrogen, in any one season. The Research Station data however do not fall into line with this statement.

The nutrient data of Tables XIX to XXII have been also calculated to a pounds of nutrient per acre basis using the factor 13.227 for converting grams per plant to pounds per acre, assuming that the rate of planting is 6,000 plants per acre. Data are set out for the whole plant only in order to save space. In Table XXIII the results of these calculations are given for the two crops grown on the medium sand.

TABLE XXIII. NUTRIENT STATUS, EXPRESSED IN POUNDS PER ACRE, OF PLANTS GROWN ON MEDIUM SAND, 1945-46 AND 1946-47

Season.	Date of Sampling	Soluble Ash lb.	Lime CaO lb.	Magnesia MgO lb.	Phosphoric Acid P_2O_5. lb	Potash K_2O lb.	Nitrogen N lb
1945-46	3/12/45*	1.3	0.2	0.05	0.08	0.5	0.2
	16/1/46	72.1	14.0	3.4	3.2	23.7	13.9
	23/1/46	132.8	28.3	6.6	6.0	42.6	26.5
	30/1/46	170.6	36.6	8.3	7.3	59.3	32.5
	6/2/46	206.0	41.1	9.3	9.0	73.2	36.2
	11/2/46	249.5	48.9	12.0	10.3	88.8	44.1
	18/2/46	249.1	54.7	14.0	11.4	84.8	50.1
	25/2/46	270.1	59.9	15.2	13.6	95.8	52.5
	5/3/46	203.8	41.9	12.2	10.5	72.9	39.0
	12/3/46	221.8	42.6	12.3	11.6	78.3	38.1
	26/3/46	264.3	46.3	13.0	15.0	100.8	46.2
1946-47	3/12/46*	1.1	0.2	0.05	0.06	0.35	0.18
	15/1/47	51.6	10.1	2.1	2.1	17.6	7.1
	29/1/47	152.8	31.3	7.8	6.8	52.4	30.7
	6/2/47	227.4	50.4	10.0	9.9	75.1	41.9
	11/2/47	253.0	54.9	12.8	11.9	94.4	50.1
	24/2/47	238.2	49.5	12.4	11.2	85.1	41.8
	10/3/47	220.1	43.7	11.8	12.3	83.3	34.9
	19/3/47	181.2	34.0	10.7	10.5	68.8	32.1
	27/3/47	178.0	32.1	10.3	10.1	60.8	32.3

* Date of planting in the field.

These data show that when the plants contained the maximum amount of nutrients, on 25th February, 1946, and 11th February, 1947, these corresponded in both seasons to approximately 55 lb. of superphosphate, 200 lb. of sulphate of potash and 250 lb. of sulphate of ammonia per acre. The total quantity of nutrients absorbed throughout the season can be obtained by combining the data for individual separates at each harvest. This is considered later in conjunction with the changes occurring in nutrient status during the harvesting period.

In Table XXIV are given data for the nutrient status of the plants on the heavier soil for the seasons 1945-46 and 1946-47, expressed in pounds per acre.

TABLE XXIV NUTRIENT STATUS, EXPRESSED IN POUNDS PER ACRE, OF PLANTS GROWN ON SILT LOAM, 1945-46 AND 1946-47

Season	Date of Sampling	Soluble Ash lb	Lime CaO lb	Magnesia MgO lb	Phosphoric Acid P_2O_5 lb	Potash K_2O lb	Nitrogen N lb
1945-46	3/12/45*	1.3	0.2	0.05	0.08	0.5	0.2
	16/1/46	40.1	7.9	2.1	1.5	13.8	9.5
	30/1/46	114.3	25.5	6.1	3.7	40.3	25.7
	11/2/46	214.4	47.9	12.2	6.5	66.4	41.4
	25/2/46	226.7	53.2	15.2	9.5	68.8	47.2
	12/3/46	196.9	43.9	13.1	7.5	54.8	44.1
	26/3/46	234.7	52.1	15.5	10.0	72.4	52.5
	9/4/46	287.4	56.0	16.9	14.6	90.3	63.5
1946-47	3/12/46*	1.1	0.2	0.05	0.05	0.35	0.2
	29/1/47	43.2	9.1	2.2	1.2†	14.8	9.8
	11/2/47	118.3	26.1	6.8	3.1	39.2	25.7
	24/2/47	210.2	45.4	11.6	5.7	72.0	44.8
	10/3/47	306.9	68.4	18.7	9.5	101.3	69.4
	27/3/47	264.0	61.0	18.3	8.1	81.3	58.6
	2/4/47	255.7	58.6	16.4	8.7	75.8	74.9
	9/4/47	255.2	58.9	17.3	11.5	72.9	55.8
	16/4/47	227.1	48.8	16.4	10.3	69.2	57.9

* Date of planting in the field. † Leaves only.

The data of Table XXIV show the greater amount of nutrients in the plants in the second season as compared with the first at the time of the harvest, the increases per acre being 15.2 lb. of CaO, 3.5 lb. of MgO, 32.5 lb. of K_2O and 22.2 lb. of N; the same quantity of phosphoric acid was present in both seasons. At all later harvests of the second season, except that on 16th April, there were greater quantities of nutrients in the plants than in the first season. The data for the final harvests show that appreciable quantities of nutrients were available for fertilizing the soil if the plants were disced in or ploughed down after harvesting was completed.

Discussion :

Under Nelson conditions the first harvests are taken from the tobacco plants while they are still growing rapidly. In the present experiments topping has usually been carried out either on the day of the first harvests or at a date very close thereto. The remaining leaves then develop rapidly as also do the stalks. The magnitude of these changes in respect of development of dry matter and of further intake of plant nutrients in the seasons 1945-46 and 1946-47 will now be considered. From the detailed data of tables XI to XIV the increases in dry matter of the whole plant, of all leaves and ripe leaves only, between the periods set out, can be calculated. Data for both soil types and for both experimental seasons are given in Table XXV. Precipitation has also been included.

TABLE XXV. SHOWING DRY MATTER INCREMENTS IN POUNDS PER ACRE BETWEEN THE STATED PERIODS

Soil type.	Period	Rainfall in.	Increment of dry matter for whole plant lb.	Increment of dry matter for all leaves lb	Increment of dry matter for ripe leaves lb.
Medium Sand	11/2/46-18/2/46	0.49	375.9	261.2	−55.6
	18/2/46-25/2/46	0.00	743.2	409.7	139.7
	25/2/46- 5/3/46	1.25	45.1	152.6	−59.2
	5/3/46-12/3/46	1.06	380.0	80.7	44.2
	12/3/46-26/3/46	0.91	1064.2	67.1	67.1
	Totals	3.71	2608.4	971.3	- -
Silt Loam	25/2/46-12/3/46	2.31	−57.0	−117.5	15.1
	12/3/46-26/3/46	0.91	532.1	120.1	69.3
	26/3/46- 9/4/46	8.09	1094.3	74.0	74.0
	Totals	11.31	1559.4	76.6	-
Medium Sand	11/2/47-24/2/47	1.90	664.5	470.0	46.8
	24/2/47-10/3/47	0.00	563.5	220.6	−50.4
	10/3/47-19/3/47	0.00	6.3	−85.6	−36.8
	19/3/47-27/3/47	2.19	201.1	107.0	48.3
	Totals	4.09	1435.4	712.0	- -
Silt Loam	10/3/47-27/3/47	2.19	305.5	136.8	13.3
	27/3/47- 2/4/47	0.00	187.0	−52.8	15.0
	2/4/47- 9/4/47	1.00	456.2	353.9	107.0
	9/4/47-16/4/47	4.53	305.4	−18.6	−10.2
	Totals	7.72	1254.1	419.3	- -

Note : The medium sand was irrigated on 15th and 30th January, 1946, and on 29th January, 1947, water equivalent to approximately one inch of rainfall being applied on each occasion. The silt loam was not irrigated in either season.

The data of Table XXV have been given in pounds of dry matter per acre rather than as grams per plant. It is clear that very large increments of dry matter occurred at certain periods in both seasons, especially just after harvesting began, because the plants were still in an actively growing condition, and at the end of the season when rains after periods of dry weather caused the development of large amounts of lateral growth, particularly in 1946. Growth can be correlated to some extent with the rainfall ; a dry period causes a depression in growth, especially it would appear, in the ripening leaf as distinct from young leaves and the stalks. However, the effect is delayed and reduction in growth was seen only after a week or two of dry weather. Response to rainfall appeared to be more rapid. Increase in weight of " nearly ripe " leaves to the " ripe " condition appeared to be irregular, especially at the Research Station, where the effects of dry weather on the plants were likely to be more severe than on the heavier soil. This resulted also in much more rapid ripening of the leaf at the Station and thus the harvesting was completed before the end of March, whereas on the heavier soil harvesting continued until about the middle of April. It is interesting to compare the total dry matter production and the weight of harvested leaf for the crops discussed in Table XXV. The requisite data are set out below, expressed in pounds of dry matter per acre.

Soil Type.	Season	Dry Matter when harvesting began lb	Dry Matter Increment during harvesting lb	Total Dry Matter for Season lb	Ripe leaves harvested lb
Medium sand	1945-46	2453.2	2608.4	5061.6	2369.3
	1946-47	2078.2	1435.4	3513.6	1875.1
Silt loam	1945-46	2135.1	1559.4	3694.5	1366.5
	1946-47	2424.0	1254.1	3678.1	1555.0

Owing to the damp conditions on the silt loam soil the plants did not grow as well as they would on a better situated field of this type. The data therefore do not show what this soil is really capable of producing. On the present figures the yield was much below that of the medium sand ; on the latter an average of 50 per cent. of the dry matter production was taken in ripe leaves, but on the heavier soil only 40 per cent. of the dry matter was harvested. The yields obtained on the heavier soil in the 1944-45 season were probably more truly characteristic of the producing capacity of this soil type.

In a similar procedure to that used with the data for production of dry matter the figures for intake of nutrients can be assembled to show the variations during the periods intervening between successive harvests. They may be set out to show the intake by the whole plants or to show the changes in the nutrient status of leaves in passing from the " nearly ripe " to the " ripe " condition. It may be said at once that except for soluble ash and lime, the latter changes were small in most cases, and judging by the negative figure often obtained, especially after a dry period, there may even have been loss of nutrients from the ripe leaves ; dry matter production when showing a negative value was not always accompanied by an apparent loss of nutrients, while in other cases an apparent increase in dry matter production seemed to be accompanied by a loss of nutrients. To what extent the summation of experimental errors in chemical analysis and in determining dry matter production affects these small differences in nutrient status cannot be stated.

To return now to consideration of the changes in nutrient status of the whole plants during harvesting ; data to give a view of the position are set out in Table XXVI

TABLE XXVI. SHOWING CHANGES IN STATUS OF WHOLE PLANTS IN POUNDS PER ACRE OF NUTRIENT BETWEEN THE STATED PERIODS FOR THE SEASONS 1945-46 AND 1946-47

Soil Type.	Period.	Rainfall in.	Soluble Ash lb.	Lime CaO lb	Magnesia MgO lb.	Phosphoric Acid P_2O_5 lb	Potash K_2O lb.	Nitrogen N lb.
Medium Sand	11/2/46-18/2/46	0.49	40.0	18.6	4.4	2.1	4.6	9.6
	18/2/46-25/2/46	0.00	83.0	21.3	5.2	4.9	27.5	13.6
	25/2/46- 5/3/46	1.25	-6.0	3.1	0.2	-0.7	7.2	3.7
	5/3/46-12/3/46	1.06	50.5	9.5	2.1	2.5	13.3	4.2
	12/3/46-26/3/46	0.91	82.3	15.0	3.3	5.5	32.7	15.1
	Totals	3.71	249.8	67.5	15.2	14.3	70.9	38.8
Silt Loam	25/2/46-12/3/46	2.31	29.5	7.8	2.0	0.1	2.6	6.3
	12/3/46-26/3/46	0.91	84.9	22.0	6.1	4.2	27.1	19.3
	26/3/46- 9/4/46	8.09	108.3	21.5	5.8	6.2	28.8	22.1
	Totals	11.31	222.7	51.3	13.9	10.5	58.5	47.7
Medium Sand	11/2/47-24/2/47	1.90	39.9	10.5	3.1	1.4	2.5	0.5
	24/2/47-10/3/47	0.00	31.4	8.4	2.2	2.7	10.8	-0.9
	10/3/47-19/3/47	0.00	7.8	3.2	1.7	0.5	0.0	3.0
	19/3/47-27/3/47	2.19	35.3	8.0	2.1	1.9	3.7	6.9
	Totals	4.09	114.4	30.1	9.1	6.5	17.0	8.5
Silt Loam	10/3/47 27/3/47	2.19	20.2	9.6	3.8	0.6	2.3	-0.5
	27/3/47- 2/4/47	0.00	44.2	14.2	2.2	1.5	6.2	23.2
	2/4/47- 9/4/47	1.00	51.9	15.8	4.9	4.2	9.2	-9.7
	9/4/47-16/4/47	4.53	28.4	7.8	3.6	0.8	8.2	13.7
	Totals	7.72	144.7	47.4	14.5	7.1	21.3	26.7

The numerical data of Table XXVI demonstrate clearly that absorption of nutrients continued right up to the end of the season. Although the amounts of soluble ash, lime, potash and nitrogen were appreciable the absorption of magnesia and phosphoric acid was much less, but nevertheless formed an important part of the seasons' totals. As was noted in the discussion on the effect of weather conditions on the production of dry matter, dry and less dry periods were associated with small intakes, or even negative values, and substantial intakes of nutrients respectively. The large positive followed by negative, values for the second and third entries of 1946 on the medium sand are possibly due to abnormally large plants having been selected for samples on 25th February ; the dry matter figures for leaves on this date appear to be unusually high.

Combining the totals of Table XXVI with the total intake of nutrients as at the first harvest the following data, expressed to the nearest pound per acre, are obtained :

Location.	Season.	Soluble Ash lb.	Lime CaO lb.	Magnesia MgO lb.	Phosphoric Acid P_2O_5 lb.	Potash K_2O lb.	Nitrogen N. lb.
Medium Sand	1945-46	499	116	27	25	160	83
	1946-47	367	85	22	18	111	59
Silt Loam	1945-46	449	104	29	20	127	95
	1946-47	452	116	33	17	123	96

On the medium sand only about two-thirds as much of the nutrients was absorbed by the plants in 1946-47 as in 1945-46, but on the silt loam the amounts were similar in both seasons. In 1945-46 the quantities

Table XXVII. Chemical Composition of Cured Leaf from Successive Harvests, Tobacco Research Station, 1945-46 Season

Constituents expressed as percentage on sand-free dry matter basis

Date of Sampling.	Soluble Ash.	In-soluble Ash	Lime CaO.	Magnesia MgO	Phos-phoric Acid P_2O_5.	Potash K_2O	Total Nitrogen N	Glucose	Fructose	Sucrose	Total Sugars.	Ratio, Total Sugars:Total Nitrogen.
Blades												
12/2/46	13.07	0.54	4.88	0.91	0.37	2.03	1.59	16.84	7.07	2.59	26.50	16.7
18/2/46	11.83	0.36	4.54	0.80	0.39	2.03	1.59	16.32	6.29	4.24	26.85	16.9
26/2/46	8.89	0.25	3.12	0.67	0.40	1.76	1.88	21.45	6.53	2.00	29.98	15.9
5/3/46	12.04	0.12	4.30	0.93	0.70	1.36	3.05	14.92	0.82	0.20	15.94	5.2
13/3/46	8.25	0.09	2.86	0.50	0.44	1.55	1.98	18.16	11.89	2.51	32.56	16.4
3/4/46	10.82	0.30	4.06	0.80	0.57	1.59	1.77	16.36	8.36	2.00	26.72	15.1
Midribs												
12/2/46	21.21	0.34	4.15	1.09	0.60	7.35	1.78	9.95	1.10	0.30	11.35	6.4
18/2/46	19.92	0.20	3.67	0.96	0.51	7.79	1.60	9.93	2.56	1.35	13.84	8.6
26/2/46	18.31	0.13	3.18	0.96	0.52	7.59	1.32	11.58	0.63	2.05	14.26	10.8
5/3/46	18.55	0.11	4.71	1.62	0.88	4.55	2.41	5.57	0.05	0.00	5.62	2.3
13/3/46	16.58	0.08	2.78	0.81	0.59	6.63	1.82	12.91	2.22	1.17	16.30	9.0
3/4/46	18.98	0.05	4.03	1.42	0.76	5.63	2.20	9.19	1.31	1.28	11.78	5.3

absorbed were substantially the same on both soil types, except that rather more potash was present in the plants on the medium sand. From the two sets of data presented above, the proportion of the total season's intake that was absorbed during the harvesting season can be readily determined. It appears that in 1945-46 substantially one-half of each of the constituents estimated by chemical analysis entered the plants during this period. In 1946-47 the proportions of each constituent varied rather more widely. Thus on the medium sand one-third of the soluble ash, lime and phosphoric acid, two-fifths of the magnesia and one-seventh of the potash and nitrogen were taken up by the plants after harvesting began ; on the silt loam one-third of the soluble ash, two-fifths of the lime, magnesia, phosphoric acid and nitrogen, but only one-sixth of the potash, were absorbed over this period.

Samples of cured leaf from the plot on the medium sand were available from the successive harvests of the 1945-46 and 1946-47 seasons. Results of chemical examination of these samples are given in Table XXVII for the former and in Table XXVIII for the latter season. Mineral, nitrogen and sugar contents are reported separately for the blade and the midrib of the leaves.

In the 1945-46 season all the harvests, except that on 5th March, showed high total sugar contents, the maximum figure being 32.56 per cent. The low sugars and high nitrogen content of the sample on 5th March may be connected with effect of the 1.25 in. of rain which fell just before this date, breaking a drought of 7 weeks duration. The high nitrogen and low sugar content has given the low value of 5.2 per cent. for the sugars:nitrogen ratio. (It is to be noted however that the " ripe " leaves of the plant separates on the above date do not show this high nitrogen figure). Soluble ash content of the blades tended to decrease in passing from lower to upper leaves, as did lime, magnesia, phosphoric acid and potash. The latter constituent was low throughout the season. On the other hand nitrogen was appreciably higher in the upper than in the lower leaves. The great distinguishing differences between the blades and the midribs are the high soluble ash and potash contents of the latter ; potash in the midribs was about four times as high as in the blades. Magnesia and phosphoric acid were relatively high in the midribs. Total sugar contents of the midribs were about half those of the corresponding samples of blades ; fructose formed a smaller proportion of the total sugars in midribs than in blades. In general the type of variation shown by the harvests of the 1945-46 season was similar to that reported above for 1944-45.

pH values and ratios of weights of separated blades and midribs are available for this season. They are set out below :

Date of Harvest.	pH value.		Ratio Weight of blade:weight of midrib.
	Blade.	Midrib.	
12/2/46	5.40	4.95	4.0
18/2/46	5.35	4.90	3.7
26/2/46	5.40	5.05	4.5
5/3/46	5.40	5.00	4.2
13/3/46	5.45	4.85	4.0
3/4/46	5.50	5.05	3.4

There does not appear to be any significant variation in the pH values for successive harvests with either the blades or the midribs ; midribs are, however, distinctly more acid in reaction than their corresponding blades. Both separates are acid, the blades giving pH values

TABLE XXVIII CHEMICAL ANALYSES OF CURED LEAF FROM SUCCESSIVE HARVESTS, TOBACCO RESEARCH STATION, 1946-47 SEASON

Constituents expressed as percentage on sand-free dry matter basis

Date of Sampling.	Soluble Ash	In-soluble Ash	Lime CaO	Magnesia MgO	Phosphoric Acid P_2O_5	Potash K_2O	Total Nitrogen N	Glucose	Fructose	Sucrose	Total Sugars	Ratio. Total Sugars · Total Nitrogen.
Blades												
12 2 47	19.90	0.12	3.64	1.35	0.46	2.75	2.23	4.44	4.23	2.11	10.78	4.8
24 2 47	13.77	0.34	4.73	0.84	0.50	3.30	1.98	10.54	8.42	7.06	26.02	13.1
11 3 47	10.32	0.06	3.38	1.35	1.51	2.01	1.96	12.29	9.02	6.09	27.40	14.0
19 3 47	8.90	0.08	2.87	0.59	0.55	1.62	2.05	15.25	10.54	4.22	30.01	14.6
27 3 47	9.15	0.06	2.89	0.64	0.59	1.84	2.37	8.70	6.56	7.87	23.13	9.8
2 4 47	12.01	0.08	3.96	0.90	0.74	2.05	1.63	5.89	5.45	1.58	12.92	7.9
Midribs												
12 2 47	23.39	0.06	4.06	0.86	0.59	8.69	2.62	5.74	2.90	2.22	10.86	4.1
24 2 47	20.61	0.07	3.58	0.62	0.63	7.99	1.87	6.20	0.29	2.86	9.35	5.0
11 3 47	19.02	0.04	2.88	0.81	0.67	7.58	1.46	9.91	4.52	1.31	15.74	10.8
19 3 47	18.81	0.03	3.01	1.02	0.87	7.29	1.51	7.94	5.00	3.19	16.13	10.7
27 3 47	18.69	0.25	3.18	1.13	0.80	6.18	1.68	5.42	3.18	1.90	10.50	6.2
2 4 47	20.09	0.18	2.14	1.47	0.97	6.56	2.06	3.97	nil	1.43	5.40	2.6

of about 5.4, and the midribs of about 5.0. The ratio of dry weight of blade to dry weight of midrib shows appreciable variations, the extreme values being 3.4 and 4.5. The high values correspond to leaves just below and just above the middle of the plant. The data for pH values and ratio of blade to midrib are very similar to those found for successive harvests in the 1943-44 season (2).

Data for the six harvests of the 1946-47 season, presented in Table XXVIII, show that although the maximum figure for total sugars of 30.01 per cent. approximated to the maximum figure of the previous season there was a wider range in sugar contents in 1946-47. Leaves of the first and last harvests were low in sugars, which gave low sugar : nitrogen ratios. Other harvests showed appreciably larger sugar values. Sucrose was higher in general than in 1945-46. The seasonal variation was in fact similar to that found in 1943-44 (2). In the leaves soluble ash, potash and nitrogen were generally higher than in 1945-46. Potash was at a more satisfactory level than in the previous season. The variation in chemical composition of the midribs was also appreciable Sugar contents, except for the first sample which was unusually well-supplied with sugars, followed the changes in the corresponding blade samples. As in the previous season the soluble ash, potash and phosphoric acid were higher than in the blades Nitrogen did not show any consistent variation, the midribs being sometimes richer and sometimes poorer in this constituent than the corresponding blades. Whereas in 1945-46, the magnesia content of midribs was always greater than that of their blades, in 1946-47 the latter were the richer in the first three but the poorer in the last three samples.

GENERAL DISCUSSION OF FIVE SEASONS' RESULTS

The aim of the commercial grower is to produce tobacco satisfactory for the purposes of the manufacturer, hence the data presented above should be examined from this point of view. Haley and Reid (3) state that seed-bed plants which will produce a satisfactory crop in respect of both yield and disease resistance should have a ratio between their nitrogen and potassium contents (N/K) of approximately 0.6, presumably at the stage when they are set in the field. Examination of the data for the four seasons in which the necessary chemical data are available, either in the present paper or in the previously published report (1), shows that in only two of them did the plants have this optimum ratio between these two constituents ; in the other two the ratios were 0.4 and 0.5. If the value of 0.6 for this ratio is applicable to Nelson conditions, and of course it is not necessarily so, it seems that the plants used in these nutrient status experiments were in some cases in a sub-optimal condition. There is no field evidence however that they were so because satisfactory growth and freedom from leaf-spotting diseases was obtained in all five seasons.

Passing now to the cured leaf, the above-mentioned authors (3) state that flue-cured tobacco of the best quality contains, in the dry blade of the cured leaf, approximately 2 per cent. of nitrogen (N), at least 2 per cent. of potassium (K) and 18 to 25 per cent. of reducing sugars, and that any significant variation from these standards results in a poorer quality of leaf. They concluded that the best quality leaf was obtained when there were not less than three units of K_2O for each unit of N in the fertilizer employed in prodicing the plants. Moreover, the best quality was found when the fertilizer treatment was such that leaves from different parts of the plant contained approximately equal contents of potash. They also stated, " that potassium deficiency actually exists in any tobacco plant that contains at maturity less potassium in the lower than in the middle and upper leaves and that such deficiency is associated with a definite lack of quality in all leaves of the plant ". In a related paper (4) it is claimed that for good quality flue-cured tobacco the ratio N/K for blades should be 0.8-1.1. In most of their satisfactory samples this figure was about 0.9. Values on either side of the above range were associated with poorer or undesirable quality. Further, it was claimed that where the potassium and nitrogen were supplied to the plants in satisfactory proportions by use of suitable fertilizers the variations in climatic conditions during the growing and harvesting seasons exerted less effect on the quality of the leaf, but nevertheless " weather conditions prior to a priming may influence the composition of the leaves ". Examination of the data for cured leaf of the 1944-45, 1945-46 and 1946-47 seasons and also for the 1943-44 season (2) shows that it was only occasionally that the N/K ratio for the blades fell in the desirable range. Moreover the nitrogen and/or potassium contents often were outside the stated figure of 2 per cent. for these constituents. The variations in nitrogen and potassium contents were such as would be expected from Haley and Reid's statement (3) that in dry seasons there is low intake of potassium and that rain after a dry period can result in poor quality leaf due to a sudden absorption of nitrogen. A striking example of this is shown in Table XXVII for the material harvested on 5th March. Heavy rain (1.25 in.) fell a few days before this harvest after a long dry period. It is noticeable that the response to nitrogen uptake was much more rapid than to potassium. Sugar content of this sample was relatively low also.

Effects of seasonal conditions noted in the above American papers (3, 4) can be traced also in the Nelson data. Thus in 1945 there appears to be a sufficiency of potash accompanied by very high sugar content of the leaf. This is characteristic of a wet season. In 1946 and 1947 the low potash content is indicative of dry conditions. The data of Gribbins *et al.* (4) indicate that two different types of variation in content of reducing sugars are to be found, one in which there is a steady decrease from the first harvest to the last, but all showing high values, and the other in which the middle leaves are much better supplied with sugars than the lower and upper leaves; thus in the latter case their middle leaves showed nearly double the sugar content of the other leaves. This type of variation occurred under Nelson conditions in the 1943-44 season (2); it is to be found also in the last experimental season of the present series of trials (see Table XXVIII). The other type of variation has been found in 1945 (Table X). In 1946, however, except for one sample, the sugar contents were all very high, the maximum figure for the season occurring at the fifth harvest. Variations in sugar content in passing from lower to upper leaves and type of season for some weeks before and during harvesting are set out below :

Season.	Conditions.	Sugar Content
1943 44	Dry, then very wet*	low -> high -> low
1944-45	Wet	very high > lower
1945-46	Dry, then moist*	high throughout
1946-47	Very dry*	low -> high -> low

* Crop was irrigated twice in 1944 and in 1946, and once in 1947, in January of each year

From this classification there does not appear to be a definite correlation between the type of variation in sugar content and the weather conditions other than that dry conditions in both December and January tend to produce the low —> high —> low type. Use of irrigation water may have complicated the indications however. Further inquiry into this matter appears to be desirable in future seasons.

Yield is an important consideration in growing a crop commercially. It is of interest therefore to see how yield of harvested leaves varied in the different seasons. The most complete data available relate to crops grown on the medium sand; they are set out below:

Season	Strain	Dry Matter lb. per acre
1942-43	Narrow-leaved	1222
1944-45	,, ,,	1231
1945-46	Broad-leaved	2369
1946-47	,, ,,	1875

The narrow-leaved strain has given appreciably lower yields than the broad-leaved one; moreover 1944-45 was a poor growing season. By courtesy of the Director of the Tobacco Research Station the weights of cured, graded saleable leaf from each harvest in 1946 were made available; from these the yield for the season was calculated to be 1973 lb. per acre. This is appreciably lower than the 2369 lb. given above and higher than the yield of 1710 lb. per acre from a neighbouring experiment in which the same fertilizer treatment was given as that for the nutrient intake experiment. A number of factors such as loss of dry matter during curing, varying percentages of moisture in cured leaf, and loss from low grade unsaleable leaf must be taken into account when considering the above differences. It is clear however that the broad-leaved strain is potentially one of high production characteristics, the yield even in the very dry 1946-47 being good.

One of the matters left for further investigation after the first two seasons' work was the confirmation or otherwise of the intake of nutrients and development of dry matter over the period of harvest. It can now be said that in each season a considerable proportion of the total intake has been absorbed by the plant after harvesting began. In general between one-third and one-half is taken up over this period. It is clear too from the detailed figures that absorption of nutrients generally continued when " nearly ripe " leaves passed to the " ripe " harvestable condition. At any one sampling date " ripe " leaves usually had a lower dry matter content than " nearly ripe " leaves; but in passing to the " ripe " condition there was often a decrease in dry matter content. Changes in concentration of minerals and nitrogen also occurred. In general, lime content increased while potash and nitrogen contents decreased; magnesia and phosphoric acid varied only slightly in most cases between the two maturity conditions.

During all the previous discussions of the chemical data no reference has been made to the insoluble ash figures which appear in the tables. This insoluble ash is a difference figure derived from the crude acid-insoluble residue from the total ash before and after treatment with dilute sodium hydroxide (5). It represents silica believed to be in the plant structure. From the tables already presented it is apparent that in some seasons, such as in 1945-46, appreciable amounts of structural silica may be present. It is not wise however to put too much emphasis on any suggested explanations for such variations as appear in the data.

Although two different strains of Harrison's Special variety have been used, one in three seasons and the other in two, it is of interest to set out the amounts of dry matter and nutrients in the unharvested leaves, laterals and stalks at the time when harvesting ceased. Data for these constituents are shown below, all being expressed as pounds per acre, and referring to crops grown on the medium sand of the Tobacco Research Station:

Season.	Dry Matter.			Nutrients				
	Leaves etc. lb.	Stalks lb	Total lb.	CaO lb	MgO lb.	P_2O_5 lb	K_2O lb	N lb
1941-42	318	1253	1571	17.1	6.8	9.8	47.2	27.8
1942-43	231	885	1116	13.5	5.1	6.4	35.0	14.2
1944-45	306	806	1112	16.0	5.4	5.4	40.5	18.5
1945-46	951	1602	2553	31.4	9.3	12.2	89.8	37.3
1946-47	334	1216	1550	22.5	7.6	7.8	48.0	20.9
Averages	428	1152	1580	20.1	6.8	8.3	52.1	23.7

If the somewhat unusal figures of the 1945-46 trial, due to the phenomenal growth of laterals in the second half of March in that season, are excluded, there are no very great variations for a given constituent from one season to another. In one season lime and potash may be relatively high and in another potash and nitrogen. The 1945-46 figure is of value however in demonstrating what may happen in a season with a good autumn rainfall after a dry summer. Taking the average figures for the five seasons the nutrients available represent per acre, 36 lb. of calcium carbonate, 36 lb. of dolomite, 40 lb. of superphosphate, 108 lb. of sulphate of potash (48 per cent. K_2O) and 116 lb. of sulphate of ammonia. The two latter fertilizer materials are expensive; consequently the addition of the equivalent of approximately 1 cwt. of each per acre in the crop residues is particularly valuable. Of the dry matter the stalks form the bulk, amounting on the average to 1152 lb. per acre ; if disced in or ploughed down in the fresh state stalks will rapidly decompose in the soil and help to maintain the organic matter content of the soil. The 428 lb. of leaves and laterals will also readily break down in the soil. On the heavier soil used in the trials the return of nutrients will be somewhat greater, especially in respect of potash and nitrogen, than on the lighter soil of the Research Station. Return of organic matter may be expected to be greater also. Altogether therefore it is to the advantage of the grower to get these residues into his soil as soon as possible after harvesting is completed. In any case this should be done before the plants are damaged by frost and other adverse conditions.

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A TEST OF THE COMBING PERFORMANCE OF WOOL, SHIPPED AFTER SCOURING.

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Summary

This processing trial was designed to test the contention of some English top makers that colonial wools, when scoured before shipment, are unsuitable for combing because baling and transport result in entanglement and felting of the wool and so cause an unsatisfactory tear in combing.

Three quarters of a lot of 3,224 lb. greasy weight of sorted 58's warp quality wool was scoured and divided into three parts One part was baled, double-dumped and shipped the return journey from New Zealand to England, one part was baled, double-dumped and held at the mill, and the third part was loosely packed and held at the mill. The unscoured quarter was baled, double-dumped and shipped the double journey along with the double-dumped scoured wool and finally scoured on its return. Thus there were four lots for the subsequent combing test.

Each lot was processed into tops by identical procedures except that the double-dumped scoured wool was given an extra passage through the willey. Measurements were made on each lot with respect to tear ratio, fibre length of top and card sliver and the nep count of the card sliver. Neither these measurements nor the observed performance from carding to combing revealed any differences of industrial importance between the lots of wool treated in the four different ways prior to processing.

INTRODUCTION

GREASY wool production in New Zealand amounts to between 120,000 and 140,000 tons annually, and of this quantity, more than 90 per cent. is shipped overseas for processing. The average yield of clean scoured fibre in the clip is probably between 65 and 68 per cent. (cf. Henderson and McMahon, 1947), so that shipment in the grease involves the carriage of over 38,000 tons of unwanted impurities long distances by sea and other transport. One of the objections raised to carrying out the initial stages of processing in the Dominion is the suggestion that scoured wool suffers felting during transit, resulting in increased fibre breakage during carding and combing, and decreasing the proportion of " top " obtained.

Modern work suggests that the principal cause of felting is not the interlocking of cuticular scales of adjacent fibres brought about by pressure (de Witt, 1888), but rather entanglement which occurs when the fibres " creep in much the same way as a worm crawls " (Arnold, 1929). " Creeping " is made possible by directional effects due to the scales and by the unique elastic properties of the wool fibre. It is facilitated by the softening effect of moisture, by high acidity or moderately high alkalinity, and can only take place when the fibrous mass is subjected to movement with alternate application and release of stress to individual fibres, such as occurs during washing, milling and felting. Under these circumstances, it is clearly most unlikely that fibre creep can occur in a dry mass of scoured and rinsed wool, tightly pressed in the wool bale and then double-dumped and secured with wire or iron bands. If wool scoured before shipment does in fact give inferior combing results, it is much more likely to be due to fibre damage caused by treatment during scouring, or to a certain degree of felting having occurred in the scouring bath, where conditions are such that this could readily take place. At the same time, the possibility must not be excluded that disappointing combing results in mills accustomed to scouring their own material have been due to insufficient appreciation of the behaviour of fibres subjected to constraint for periods of several months. Although wool is one of the most elastic of our textile materials, deformed fibres do not always return to their original relaxed dimensions immediately the cause of deformation is removed. Rinsed wool subjected to the scouring process is brought to the card in a completely relaxed and lofty state, whereas wool which has been pressed after scouring requires more opening, preferably in a damp atmosphere, to release the temporary sets which the fibres have sustained

THE RAW GREASY WOOL

A sorted commercial lot of New Zealand wool in the grease, 58's combing warp quality, total weight 3,224 lb., yielding 67.3 per cent., and supplied by the Bruce Woollen Manufacturing Co., Ltd., Milton, New Zealand, was chosen for the test.

TREATMENT PRIOR TO THE PROCESSING TRIAL

General Scheme.

Sorted Wool, 3,224 lb., greasy

Scoured $\frac{3}{4}$ of total and packed into bales

$\frac{1}{4}$ of total packed into two bales in the grease

2 bales double-dumped and shipped N.Z. to England and return. (Lot 1)	2 bales double-dumped and held at mill. (Lot 2)	Bales loosely packed and held at mill. (Lot 3)	2 bales double-dumped, shipped N.Z. to England and return and finally scoured. (Lot 4)

For subsequent processing each lot was split into two parts called Batch A and Batch B, each bale forming the unit where possible.

Scouring.

The scouring of the wool was carried out in a four bowl set, three feet wide, harrow type, to meet the requirements for high quality worsted processing.

The greasy wool, after passing through a Taylor Wordsworth double cylinder willey (the front and back cylinders ran at 480 and 580 R.P.M. respectively), was fed to the scouring set by means of an automatic feeder at about 420 lb. per hour. The first two bowls were each of 1,900 gallons and contained respectively 0.15 per cent. and 0.04 per cent. of soap and 0.07 per cent. and 0.01 per cent. soda ash. The third bowl contained water only and fresh warm water was supplied continuously to the rinse bowl. The temperatures were 126°F. for the first and second bowls, 120°F. for the third bowl, and 100°F. for the rinse bowl. Before commencing to scour the wool the liquors were run in on merino pieces for two hours. During scouring, measurements of pH and temperature of the liquors were made at hourly intervals, and when necessary, at shorter intervals.

The scoured wool was dried in a three-tier Taylor-Wordsworth dryer operating at 150°-165°F.

In scouring the two bales of greasy wool returned from England, care was taken to give it the same treatment as was given the portion corresponding to Lots 1 and 3.

The mean results of analyses of the scoured wool are given in Table I.

TABLE I. ANALYSES OF SCOURED WOOL

Lot No.	Grease Content.		Absolute Alcohol Extract (after pet. ether). (Per cent.)	Sorbed Alkali as NaOH. (Per cent.)
	Mean. (Per cent.)	Range.		
1-3	0.69	0.27 per cent. to 1.2 per cent. on 17 tests	0.81 (mean of 3 tests)	0.34
4	0.97	0.61 per cent. to 1.44 per cent. on 7 tests	1.00 (mean of 4 tests)	0.37

Baling.

Before baling, the scoured wool corresponding to Lots 1 to 3 was allowed to lie for four weeks in bins by which time it reached an average regain of 11.9 per cent.

Standard jute packs were used for baling each lot. The bales were then treated as shown in Table II.

TABLE II DETAILS OF BALING

Lot No.	Bales.	Weight of each bale. (lb. net)	Treatment of bales.
1	2	274, 274	Double-dumped and shipped.
2	2	270, 274	Double-dumped and held at the mill.
3	6	91 (mean)	Loosely packed and held at the mill.
4	2	340, 341	Double-dumped and shipped.

Lots 1 and 4, after double-dumping, were shipped to England per the "Antar" and returned to New Zealand per the "Rimutaka". All the lots remained in bales for seventeen months with the exception of Lot 4 (fourteen months) before further processing, Lots 1, 2 and 4 being in double-dumped form.

PROCESSING TRIAL

For subsequent processing each lot was divided approximately into halves, taking a bale for each unit in the case of Lots 1, 2 and 4, thus giving eight batches designated as 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B. Processing was carried out in the following batch order: 3A, 2A, 1A, 4A, 4B, 1B, 2B, 3B.

All the machinery used was made by Taylor Wordsworth and Co., Ltd., and suitable for processing fine crossbred wools.

Opening.

Each batch was first put through a double cylinder willey or opener of the following characteristics :—The cylinders were 28 in. in diameter, studded with spikes 2½ in. long, and of effective width 28 in. The nip of the fluted feed roller could not close to less than ¼ in. and was located 2½ in. from the tips of the spikes of the first cylinder. The speeds of the first and second cylinders were respectively 383 R.P.M. and 453 R.P.M. and the surface speed of the feed rollers was 22.7 ft. per minute.

After observing that the rates of carding production of Batches 1A and 2A were greater (most probably due to compact pieces of wool falling into the weigh pan of the automatic feed and reducing its precision of weighing) than for the other batches, the decision was made to increase the degree of opening of Batches 1B and 2B by a second passage through the willey.

Carding.

A medium worsted card, 60 in. wide, of conventional design and with automatic feed was used, the speed of the swifts being between 109 and 112 R.P.M.

In preparation for the trial, the card clothing was ground and run for six days followed by fettling and a further run for two days on 60's wool. The card was again fettled and run-in between Batches 4A and 4B.

Before feeding to the card the opened wool was weighed, regain determined, and oiled on the floor with a stirrup pump using an emulsion of batching oil (JB/QSX by Benj. R. Vickers and Sons Ltd.) calculated to give ¾ per cent. added oil and 24 per cent. mean regain on the wool.

The automatic feed to the card was set to give an average output of 60 to 64 lb. per hour and remained unchanged throughout the trial.

The carding production was measured by weighing the contents of each can as it was taken off at 15 minute intervals. The temperature and relative humidity of the atmosphere at the card was measured by a wet and dry bulb whirling hygrometer at 90 minute intervals.

Samples of card sliver, each about 9 feet long, were taken during carding from can Nos. 3, 8 and 14 in order of production for measurements of fibre length and nep count.

Backwashing to Punchballing.

The card sliver was backwashed in a two-bowl machine, with a 9 cylinder dryer, and a double-headed machine delivering into cans. A 0.13 per cent. soap liquor was used in the first bowl and rinsing water in the second, the bowls being made up fresh for each batch. The average liquor temperatures were 110° - 115°F. and the drying temperature about 152°F. yielding a sliver at approximately 22 per cent. regain at the backwash gill where 3 per cent. of batching oil (Vickers JB/QSX) was added.

The slivers from the backwash gill were passed through one strong box and on to the punch box where 13 lb. balls were made, except that in Batch 4B the balls were 12¼ lb. The drafting data are given in Table III.

Combing.

In combing by the Noble comb, care was taken to ensure that the settings of the feed knives and the draw-off rollers remained the same for processing each lot. The feed knives were kept set in one position. The dabbing brushes were in good condition.

Measurement of the tear ratio was carried out by weighing the sliver and noil corresponding to groups of three cans and single cans in succession, but rejecting the last three cans with the corresponding noil because of possible irregularity due to the punch balls beginning to run out.

From the second finishing gill, a top sliver of 5 oz. per 10 yd. was delivered in balls of mean weight 8 lb. 2 oz. The drafting data are given in Table III.

TABLE III. DRAFTING DATA

Operation	Weight of Sliver fed to Machine. (oz./10 yd.)	Number of Ends up.	Draft
Backwash gill	10.0	10	7.8
Strong box	12.8	5	6.8
Noble comb	9.4	72	-
1st Finishing gill	2.6 approx.	16	5.75
2nd Finishing gill	7.2 ,,	4	5.8 approx.

Samples of top for fibre length measurement were taken from four balls distributed over each batch.

RESULTS AND DISCUSSION

State of the Scoured Wool.

Batches 1A and 1B smelled a little musty in the centres of each bale after breaking open, but the appearance of the wool under ultra-violet light or under the microscope or after staining with methylene blue did not differ from the other batches.

Opening.

The higher carding production rate for Batches 1A and 2A shown in Table VI indicated a lower degree of loftiness for these compared with the other willeyed lots and this was also apparent to the eye. After passing Batches 1B and 2B twice through the willey this difference was no longer apparent and the carding rate was restored to normal.

The weights of sweepings from under the willey are shown in Table V. In view of the variable nature of these impurities it is considered that the differences between the figures have no importance.

Carding.

All lots ran satisfactorily.

The atmospheric conditions at the card are shown in Table IV.

TABLE IV. ATMOSPHERIC CONDITIONS AT CARD

Batch No.	Temperature in °F. at			Per Cent. Relative Humidity at		
	15 min.	105 min.	240 min	15 min.	105 min.	240 min.
1A	77	77	77	51	49	49
1B	72	76	—	49	51	--
2A	83	83	82	48	53	53
2B	82	82	77	48	50	52
3A	72	86	86	56	46	47
3B	79	79	77	57	45	43
4A	75	78	78	60	53	53
4B	74	71	78	43	41	38

The temperatures during carding would all be regarded as satisfactory but the humidity of the atmosphere was rather low while carding Batch 4B and part of Batch 3B. No effects of static electricity were noticed however.

Card waste collected as sweepings from under the card is recorded in Table V. Again the variable nature of the sweepings is the reason for regarding the differences between the figures as of no importance.

TABLE V. WILLEY AND CARD WASTE

Batch No	Weight of Batch after opening	Sweepings			
		Willey.		Card	
	lb	lb.	oz	lb	oz.
1A	280	0	10	0	8
1B	276	1	7	0	10
2A	281	1	4	0	3
2B	279	1	5	0	14
3A	287	-		1	9
3B	254	1	3	0	7
4A	220	0	11	0	4
4B	220	0	11	1	2

The nep contents of the samples of card slivers were counted on foot lengths (about 10 g.) of two sliver samples from each batch by the method of Townend and Spiegel (1944) and the results are recorded in Table VI. The relatively high number of neps for Batches 4A and 1A can probably be explained as being due to the fact that these batches were the last two carded before fettling. The differences found between the other batches are not significant.

The results of fibre length measurements on the samples of card sliver by the cut-squaring-method described by Daniels (1942) are recorded in Table VI.

TABLE VI FIBRE LENGTH AND NEP COUNT OF CARD SLIVER

Batch No.	Fibre Length					Nep Count as neps per g		Carding Production (lb per hour)
	No. of fibres measured.	Mean Length. (cm.)	* S D. (cm)	* S.E. (cm)	* C of V. (Per cent.)	Separate Results	Mean	
1A	1,393	7.22	4.98	.13	68.9	25, 30	28	71
1B	1,332	6.93	4.86	.13	70.1	18, 16	17	64
2A	1,504	7.03	4.96	.13	70.6	14, 20	17	74
2B	1,838	7.02	5.03	.12	71.6	22, 21	22	63
3A	1,313	7.06	5.00	.14	70.7	15, 19	17	61
3B	1,756	7.69	5.09	.12	66.2	14, 21	18	64
4A	1,416	7.14	5.04	.13	70.6	34, 30	32	60
4B	1,407	6.98	4.90	.13	70.2	17, 23	20	61

* S.D. = Standard Deviation. S.E. = Standard Error. C. of V. = Coefficient of Variation.

The results of fibre length measurements on the card slivers are shown graphed as cumulative diagrams (corresponding to Baer diagrams) in Fig. 1 which shows the " B " series only. Curves for series " A " are not shown but they follow closely those for 1B, 2B and 4B.

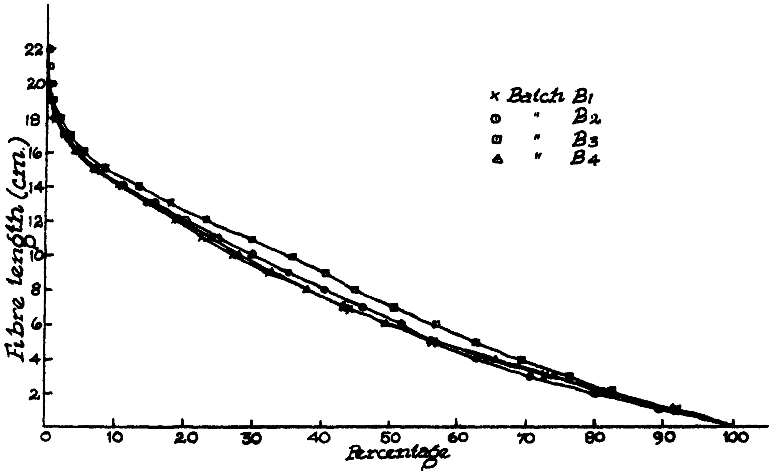

FIG. 1

The mean fibre length of the card sliver of Batch 3B is significantly longer than for the other batches and could be of practical importance, but it is not confirmed by its duplicate Batch 3A nor is it supported by Batches 4A and 4B. Although the difference in the mean fibre length between Batches 1A and 1B is statistically significant it is considered too small to be of practical importance. The other differences in mean fibre length are not significant.

Combing.

The cut-squaring-method of Daniels (1942) was also used for determining the fibre length of the tops, the samples being taken from four balls distributed over each batch. These results, together with the tear ratios, are shown in Table VII. Results for Batches 1A, 2A, 3A and 4A are not given because, owing to a misunderstanding, a gilling was omitted before combing. The fibre length measurements are shown plotted as cumulative diagrams in Fig. 2. Again the fibre length measurements have failed to reveal, with the exception of Batch 3B, any

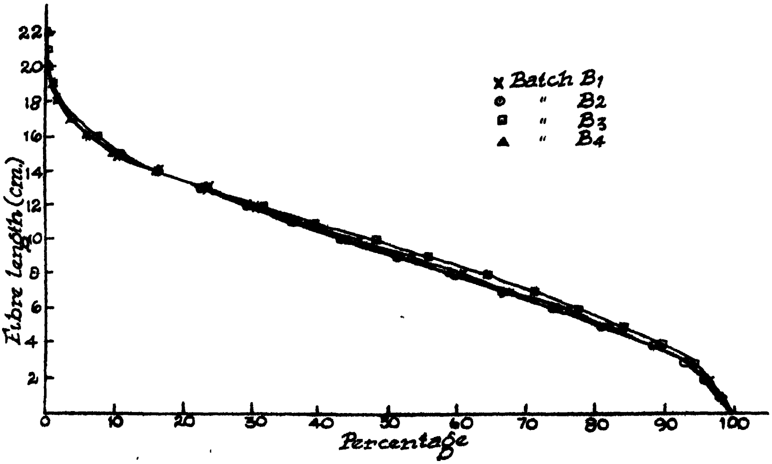

FIG. 2

differences of practical significance between the lots. The slightly greater mean fibre length of Batch 3B is supported by the results of measurements on the card sliver but is not supported by corresponding measurements on Batch 4B. Possibly the fact that Batch 3B was the last one processed contributed to its slightly superior performance.

Included in Table VII are the results of fibre-fineness measurements on the tops under the projection microscope. The fineness figures do not appear to warrant comment other than that the quality number of the tops approximates 56's on the Bradford scale.

No importance can be attached to the differences shown between the figures for the top to noil ratios, which, from an analysis of the weights measured during combing, are not statistically significant.

TABLE VII. TEAR, FIBRE LENGTH AND FIBRE DIAMETER OF TOPS

Batch No	Top to Noil Ratio.	Fibre Length					Fibre Diameter.			
		No. of Fibres.	Mean Length (cm.)	S.D. (cm.)	C. of V. (Per cent)	S.E. (cm.)	Mean Diam. (μ)	S.D. (μ)	C. of V. (Per cent.)	S E (μ)
1B	6.05 : 1	1602	9.42	4.31	45.8	0.11	28.9	6.4	22.2	0.23
2B	6.43 : 1	1677	9.29	4.39	47.3	0.11	28.7	6.5	22.6	0.29
3B	6.09 : 1	1689	9.66	4.29	44.4	0.10	28.3	6.1	21.6	0.28
4B	6.21 : 1	1987	9.33	4.39	47.1	0.10	27.9	6.5	23.2	0.24

CONCLUSIONS

It is considered that no differences of industrial importance were found in the carding and combing properties of the wool irrespective of whether it was scoured and double-dumped before shipment, double-dumped in the grease before shipment, scoured and stored after double-dumping, or scoured and stored loosely packed.

ACKNOWLEDGMENTS

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POLLEN IN HONEY AND BEE LOADS

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Summary

Samples of honey and bee loads obtained from test hives situated within "bee range" of native bush, scrub and pasture, at Pongakawa, New Zealand, were examined for their pollen content. It was found that during the season November, 1945, to March, 1946, the pollen of only a few of the species in this area, which are known to be visited by honey bees, was recovered in the honey and bee loads. Most of the pollen in the honey came from scrub (manuka), pasture and waste places (clover, lotus, catsear, hawkbit, and thistle). Native bush was represented by a small percentage of rewarewa. The principal pollen types in the bee loads were from pastures and roadsides (lupin, clover, thistle, lotus, catsear, hawkbit, and plantain).

INTRODUCTION

It is desirable to know to what extent different honey types are characterized by their pollen content. The source of the parent nectars cannot be deduced from the pollen content of the honey without reference to other characters and to methods of extraction, but cases do arise in which it is desirable to identify the pollen in honey. Under suitable conditions much can still be learned concerning the activity of honey bees at different seasons in particular localities, from the study of the pollen content of honey samples and bee loads. The present paper deals with a series of honey samples and bee loads which were examined in connection with the botanical aspects of a recent outbreak of honey poisoning investigated by the Department of Agriculture. A general description of the area, and of the manner in which the samples were obtained, is given elsewhere (Harris and Filmer, 1947, and Paterson, 1947).

The sources of honeys in New Zealand were discussed by Cockayne (1916), and more recently by Winter (1946), and have also been the subject of numerous articles concerning particular localities. However, little published work exists concerning the pollen in New Zealand honeys. Waters (1915-6) gave detailed descriptions of a few pollen types, but pollens of indigenous plants were not dealt with. Since Waters' descriptions were published, his methods of preparation have been superseded by methods evolved in the northern hemisphere, where considerable attention has been given to pollen morphology, and to the identification of pollens in peat, on atmospheric slides and in honey. The only available key for the identification of the pollens of indigenous plants (Cranwell, 1942), is based on these more recent methods of preparation, but lacks illustrations and is complicated by the inclusion of many pollen types unlikely to concern the investigator of honey pollens. There is therefore, little information available concerning pollen types in New Zealand honeys, which is based on the newer methods of preparation, and covers both exotic and indigenous species.

POLLEN AND NECTAR FLORA

The apiary was situated in the Pongakawa Valley where native bush, scrub and pasture were within " bee range " of the hives. A botanical survey was carried out to determine the species available to bees in this area, and nearly 200 were noted (see Appendix). A relatively large proportion of these (about two-fifths) was cultivated, in many cases represented by only a few individual plants. The scrub contained fewer species, which, however, were more abundant. In the small area of bush examined some 40 species were observed, mostly trees and shrubs, of which only a few were entomophilous, and none were abundant. One third of the species listed were weeds and herbs occurring in pastures, on roadsides, or in waste places, mingling at times with the scrub. Some few of these were sufficiently abundant to be of importance to bees, as the pollen results show. Sixty-one of the species recorded are known to be worked by bees for pollen, and 150 species are known to be nectar plants, 66 of which have been reported to be visited by bees.

MATERIALS

(*a*) *Honey* :

The honey and pollen samples were collected by Mr. C. R. Paterson, Apiary Instructor, Department of Agriculture, Hamilton, and forwarded for investigation.

To secure samples of incoming honey collected over definite periods the following procedure was adopted. " Prior to the commencement of the tests the bees were allowed to use up all stored honey. Sugar syrup was then fed to sustain them until the first spring nectar was available. To ensure that a sample of honey covering a definite period would be secured, hives A and B were both kept overcrowded and one or two empty drawn-out combs were placed in an appropriate position in the hives and the date noted. After samples had been cut out from these combs covering a period of approximately 14 days they were replaced by fresh empty combs. In this way it was considered that a complete coverage of all incoming nectar and pollen would be obtained." (Paterson, 1947).

A weighed amount of honey, 25 or 50 g., was dissolved in warm water and the pollen content concentrated by centrifuging. Pollen was either stained with basic fuchsin or treated by Erdtman's " acetolysis " method (Erdtman, 1943).

(*b*) *Bee Loads* :

" Samples were secured by placing a pollen trap at the entrance of hive B. The bees in this hive were of a black strain, while those in hive A were Italian. The bees very soon became accustomed to forcing their way through the trap, the pollen being knocked off their legs in the process, and collected in a tray at the bottom." (Paterson, 1947).

The pellets were mostly lenticular in shape, and various colours. The pellet size and weight were less variable than the colour, for most of the pellets were about 3 mm. long, and weighed about 1 mg. (air dry). From each sample of bee loads a random lot of over 200 pellets was taken for detailed examination. These pellets were grouped according to their colour, and each colour group was examined for consistency of pollen type, and sorted accordingly. The percentage of each pellet type in the bee load sample was then estimated.

IDENTIFICATION OF POLLENS

Identifications were made by reference to keys*, illustrations†, and a set of pollen slides prepared from the species noted in the botanical survey of the district.

It is not always practicable to separate pollens of closely related plants, so for statistical purposes we have designated these pollen types by the vernacular name of one of the common species each represents. However, the application of this rule varies according to circumstances, as species not known to occur in the locality were not taken into consideration.

The following are the principal pollen types encountered in this study.

IMPORTANT POLLEN TYPES IN HONEY AND BEE LOADS

POLLEN TYPE	SPECIES REPRESENTED
Catsear	*Hypochaeris radicata* L. (catsear), *Leontodon hispidus* L. (hawkbit).
Clover	*Trifolium* species—*T. pratense* L., *T. repens* L.
Lotus	*Lotus uliginosus* Schkuhr. (*L. major* Sm.).
Lupin	*Lupinus arboreus* Sims.
Manuka	*Leptospermum scoparium* Forst., *L. ericoides* A. Rich. (and possibly other *Myrtaceae*).
Thistle	mostly *Cirsium lanceolatum* (L.) Hill.

POLLEN TYPE	SPECIES REPRESENTED	HONEY	BEE LOADS
Buddleia	*Buddleia* spp.	—	+
Caryophyllaceae	All species	+	—
Compositae	All species	+	—
Conifer	Pines, etc.	+	+
Ericaceae	All species	+	+
Grass	All species	+	+
Malva	*Malva* spp.	+	+
Pigeonwood	*Hedycarya arborea* Forst.	—	+
Plantain	*Plantago lanceolata* L.	—	+
Tutu	*Coriaria arborea* Lindsay.	+	—

POLLEN RESULTS

(*a*) *Pollen content of honey :*

There are acknowledged difficulties in correlating quantitatively, or even qualitatively, the pollen in honey with the sources of nectar (Melville, 1945). It is remarkable, however, that the pollen results are fairly consistent over a period, whereas, if there were no relationship between the proportion of pollen and of the parent nectars, a more fortuitous occurrence of the pollen maxima might be expected.

Details of the pollen content of the samples are given in Table I.

Though manuka was the predominant pollen throughout the period studied, and though there was also a small but constant percentage of rewarewa pollen in November and December samples, introduced species were represented by the greatest diversity of pollen types. In December there was a high proportion of clover pollen in sample (ii) taken from the hive on the 15th, which may have been caused by favourable weather conditions. The bee load sample taken on the same day was composed entirely of white clover pollen. Throughout February there was a small but constant percentage of thistle pollen, and in sample (ii) taken from the hive on 15th March, when *Cirsium lanceolatum* was in full flower, there was 46 per cent. thistle pollen.

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* Cranwell (1942), Wodehouse (1935).

† Wodehouse (1935, 1945), Armbruster and Oenike (1929).

During the botanical survey of the area, 66 species were observed which are known to be visited by honey bees for their nectar, and 56 of these could be represented by the pollen types recognized in the honey samples.

(*b*) *Pollen content of bee loads :*

Pollen contains foods essential for the bees, and where a choice is available selection is made according to their " tastes ", the selection being based on the relative abundance of certain materials in the respective pollens. The area in which the apiary was situated provided a variety of plant communities and pollen types within " bee range " of the apiary, but the bee loads examined showed that the bees had worked only a few of the pollen types available and these principal types were most common in pastures and waste lands. Thus the main sources of pollen were, lupin in November, clover in December, lupin and thistle in January, and thistle in February. Small amounts of lotus, catsear, hawkbit, and plantain pollen were also found (see Table II).

TABLE I. PERCENTAGE POLLEN CONTENT OF HONEY SAMPLES

Date.	Sample.	Manuka	Rewa-rewa.	Clover.	Lotus.	Catsear.	Thistle.	Others.
1945								
*Feb.	Unfit for use	61	1	14	15	2	1	6
Nov.								
17	i	93	1	3	- -	- -	--	3
	ii	82	4	5	-	- -	- -	9
29	i	61	11	22	1	-	——	5
	ii	71	3	12	1	4	-	9
Dec								
15	i	76	2	15	-	2	- -	5
	ii	0	1	67	11	-	1	—
29	i	73	1	9	10	-	1	6
	ii	94		1	2	-	1	2
1946								
Jan.								
7	i	87		8	5	--	- -	-
	ii	82		3	11		1	3
14	i	58	-	32	6		-	4
	ii	97	-	3	-	-	- -	——
21	i	87	- -	6	--	6	-	1
	ii	91	- -	4	1	- -	1	3
Feb.								
3	i	90		4	--	-	3	3
	ii	90	-	6	3	-	1	-
21	i	55	-	31	2		7	5
	ii	71	—	10	13	- -	6	
Mar.								
15	i	85	- -	3	3	1	7	1
	ii	17	2	15	1	18	46	1

* Sample from the same locality, included for comparison.

The differences in colour observed in pellets composed of the same pollen type may be influenced by the presence of varying amounts of wax, and/or, by the presence of other pollen types. Traces of other pollen types might be attributable to contamination, either of the flower by wind- or insect-borne pollens, or from the honey with which the pollen load is moistened. Mixed loads, containing high proportions of two or more pollen types were found, probably when certain types were in limited supply. Thus thistle was found in mixed loads early in February, but later in pure loads and in much greater abundance.

COMPARISON OF IMPORTANT POLLEN TYPES IN HONEY AND BEE LOADS

	HONEY	BEE LOADS
November	manuka	lupin
December	manuka, clover	white clover
January	manuka	lupin, thistle
February	manuka	thistle
March	manuka, thistle	thistle

TABLE II POLLEN CONTENT OF BEE LOADS

Date.	Pellet Colour.	Pollen Type.	Percentage of Pellets per Sample.
1945			
Nov. 17	Deep orange	lupin	1
	Orange	lupin	68
	Lemon	lupin	17
	Pale lemon	lotus	4
	Green-black (mixed)	thistle, lupin	10
29	Orange	lupin	93
	Lemon	lupin	5
	Pale lemon	*Buddleia*	1
	Black	thistle	1
Dec. 15	Orange	*Trifolium repens*	87
	Lemon	*Trifolium repens*	13
	Lemon to orange	*Trifolium repens*	
1946			
Jan. 7	Deep orange	lupin	15
	Orange	lupin	42
	Lemon (mixed)	lotus and thistle	31
	Brown (mixed)	lotus and lupin	8
	Grey	lotus	4
14	Deep orange	lupin	8
	Orange	catsear	1
	Lemon	thistle	79
	Brown (mixed)	lupin and lotus	8
	Grey	thistle	8
21	Deep orange	lupin	2
	Orange	catsear	4
	Lemon	thistle	86
	Brown	lupin	3
	Grey	thistle	5
Feb. 3	Deep orange	lupin	1
	Orange	catsear	12
	Light orange		
	Yellow (mixed)	thistle and monocotyledon	1
	Fawn lemon	thistle	86
	Fawn white		
	Grey-black		
21	Deep orange	lupin	13
	Orange	catsear	25
	Lemon	thistle	50
	Bright lemon	thistle	1
	Grey	thistle	8
	Blackish	thistle	3
Mar. 15	Deep orange	catsear	14
	Orange		
	Light Brown	thistle	75
	Yellow brown		
	Brown (mixed)	lupin and Myrtaceae	4
	Mixed brown	lupin and Myrtaceae	1
	Blackish	thistle	1
	Grey	thistle	4
	White	thistle	1

Some of the honey produced in this locality prior to the present investigation was said to be difficult to extract—a characteristic of manuka honey. Though there was generally a high percentage of manuka pollen in the samples examined, other species may have been more important nectar sources than the pollen statistics indicate. Rewarewa, for example, which is represented by a much smaller percentage of pollens, has a more specialized type of flower and a larger pollen. Possibly rewarewa honey might contain less pollen than manuka honey.

It is interesting to note that the flowers of *Lupinus* were described by Knuth (1908, p. 271), as " nectarless bee flowers ", for in the bee loads examined here lupin pollen was important, but very little was found in the honey.

Small amounts of pollen from plantain, pigeonwood, and buddleia were found in the pellets, but not in the honey. This indicates that the bees have worked these plants only for pollen (plantain, pigeonwood), or that pollens were present in only small numbers in the honey and were missed in sampling (***Buddleia***, Howes, 1945).

CONCLUSIONS

The above results do not afford comparisons between different localities or honey types, but the data are presented for possible future use in this connection. Should future work along these lines be indicated, a key, with illustrations and brief descriptions would facilitate recognition of the main pollen types likely to be encountered.

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APPENDIX I

THE PLANT LIST

The plant list was compiled during the botanical surveys of the area by W. F. Harris and A. J. Healy (November, 1945) and H. E. Connor (February, 1946). Literature was consulted for references to the utilization of flowers as nectar or pollen sources, with the primary object of devising a guide for the identification of pollens in honey and bee loads. In this appendix the authorities referred to are designated by numerals as indicated at the end of the list.

PLANTS OBSERVED DURING BOTANICAL SURVEYS OF THE PONGAKAWA DISTRICT

Common Name.	Botanical Name.	Nectar Flowers.	Pollen Flowers.	Worked by Bees.
Alyssum	*Alyssum* sp.	7	7	7
Anchusa	*Anchusa* sp.	7	9	7,9
Antirrhinum	*Antirrhinum majus*	6,7	7	7
Apple	*Pyrus malus*	7	7	7
Arctotis	*Arctotis* sp.			
Azalea	*Azalea* sp.	6,7		
Barberry	*Berberis vulgaris*	7,8	7,9	7,8,9
Bean, broad	*Vicia faba*	7	9	7,9
Bean, kidney	*Phaseolus vulgaris*	3		
Bedstraw, native	*Galium umbrosum*	4		
Beet	*Beta vulgaris*	6		
Bluebells, native	*Wahlenbergia gracilis*	3		
Blueberry	*Dianella intermedia*			
Bluegum	*Eucalyptus globulus*	3		3
Broadleaf	*Griselina littoralis*		4	
Broom	*Cytisus monspessulanus*			
Tree lucerne	*C. proliferus*	3		
Broomrape	*Orobanche minor*	6		
Buddleia	*Buddleia* sp.	7	9	7,9
Buttercup, native	*Ranunculus hirtus*			
Buttercup	*R. repens*	2,8	9	8,9
Bushlawyer	*Rubus cissoides*	3		
Californian poppy	*Eschscholtzia californica*	7	7,9	7,9
Canterbury bells	*Campanula* sp.	7	7,9	7,9
Cardamine, native	*Cardamine heterophylla*	6		
Carnation	*Dianthus* sp.	6	9	9
Catchfly	*Silene gallica*	1		
Catmint	*Nepeta* sp.	7		
Catsear	*Hypochaeris radicata*	3,8	3	8
Cherry, flowering	*Prunus cerasus*	7	7	7
Chickweed	*Stellaria media*	7	7,9	7,9
Chrysanthemum	*Chrysanthemum* sp.	6		
Cleavers	*Galium aparine*	4		
Clover, red	*Trifolium pratense*	7,8	9	7,8,9
Clover, subterranean	*T. subterraneum*	6		
Clover, suckling	*T. dubium*	7		7
Clover, white	*T. repens*	7	9	7,9
Columbine	*Aquilegia* sp.	2	7,9	7,9
Coneflower	*Rudbeckia* sp.	7		7
Coprosma, broad-leaved	*Coprosma australis* (*C. grandifolia*)		4	
Cornflower	*Centaurea cyanus*	2	9	9
Cudweed, purple	*Gnaphalium purpureum*	4,6		
Currant, flowering	*Ribes glutinosa*	6		
Daisy, native	*Lagenophora pumila*	6		
Dandelion	*Taraxacum officinale*	7,8	7,9	7,8,9
Delphinium	*Delphinium* sp.	2	9	9
Dock, broad-leaved	*Rumex obtusifolius*		+	
Earina	*Earina mucronata*	4		
Epilobium	*Epilobium* sp.	7	7	7
Evening primrose	*Oenothera* sp.		6	
Evonymus (spindle tree)	*Evonymus japonica*	1		
False-acacia	*Robinia pseudacacia*	3,7		3,7

Common Name.	Botanical Name.	Nectar Flowers.	Pollen Flowers.	Worked by Bees.
Fathen	*Chenopodium album*		+	
Fireweed, Australian	*Erechtites atkinsoniae*	6		
Fivefinger	*Nothopanax arboreum*	4		
Flax, Australian	*Linum marginale*	1		
Fleabane	*Erigeron crispus*	6,7		7
Foxglove	*Digitalis purpurea*	6		
Fuchsia, native	*Fuchsia excorticata*	3,8		8
Geranium, scarlet	*Pelargonium hortorum*		9	9
Gerbera	*Gerbera* sp.	6		
Geum	*Geum* sp.	1,7	7	7
Japanese cudweed	*Gnaphalium japonicum*	6		
Gooseberry	*Ribes grossularia*	7	7	7
Grape	*Vitis vinifera*	7	7	7
Groundsel	*Senecio vulgaris*	6		
Hangehange	*Geniostoma ligustrifolium*			
Hawkbit	*Leontodon hispidus*	7	7	7
Heath	*Erica* sp.	7,8	9	7,8,9
Hinau	*Elaeocarpus dentatus*	5		
Hollyhock	*Althaea officinalis*	7	7,9	7,9
Honeysuckle	*Lonicera* sp.	7	9	7,9
Honeysuckle	*Lonicera nitida*	7	9	7,9
Hydrocotyle, native	*Hydrocotyle novae-zealandiae*	6		
Ice plant	*Mesembryanthemum* sp.	7	7	7
Inkweed	*Phytolacca octandra*		?	
Ivy	*Hedera helix*	7	7,9	7,9
Jasmin	*Jasminum* sp.	6		
Jasmin, native	*Parsonsia heterophylla*	3		
Jasmin, small-flowered native	*P. capsularis*	3		
Kahakaha	*Collospermum hastatum* (= *Astelia solandri*)		5	
Kamahi	*Weinmannia racemosa*	4,8		8
Kanuka, teatree	*Leptospermum ericoides*	3		+
Karamu	*Coprosma robusta*		4	
Kiekie	*Freycinetia banksii*			
Koromiko	*Hebe salicifolia*	6,8		8
Lavender	*Lavandula* sp.	7		7
Lilac	*Syringa vulgaris*	6,7	9	6,7,9
Lily	*Lilium* sp.		7,9	9
Linaria	*Linaria* sp.	1,7		7
Lotus	*Lotus angustissimus*	6	9	9
Lotus	*L. corniculatus*	1	9	9
Lotus	*L. hispidus*	6	9	9
Lotus major	*L. uliginosus*	6,8	9	8,9
Lucerne	*Medicago sativa*	3		3
Lupin, blue	*Lupinus angustifolius*		7,9	7,9
Lupin, tree	*L. arboreus*		7,9	7,9
Mahoe (whitey wood)	*Melicytus ramiflorus*	4		
Makomako (wineberry)	*Aristotelia serrata*		4,5	
Malva	*Modiola caroliniana*	7	7,9	7,9
Manuka	*Leptospermum scoparium*	3,8		8
Mapou (matipou)	*Suttonia australis*		4	
Marigold	*Tagetes* sp.	6		
Mayweed, rayless	*Matricaria discoidea*	6		
Milkweed	*Euphorbia peplus*	1,7	7	7
Mingimingi	*Leucopogon fasciculatus*			
Muehlenbeckia	*Muehlenbeckia australis*			
Mullein	*Verbascum* sp.	7	7	7
Nemesia	*Nemesia* sp.			
Nettle	*Urtica incisa*		+	
Nightshade, black	*Solanum nigrum*		+	
Niniwa	*Gaultheria oppositifolia*	+		
Onion	*Allium* sp.	7		7
Oxalis	*Oxalis corniculata*	1		
Oxalis	*O. stricta*	1		
Oxeye daisy	*Chrysanthemum leucanthemum*	7		7

Common Name.	Botanical Name.	Nectar Flowers.	Pollen Flowers.	Worked by Bees.
Pansy	*Viola tricolor*	1	9	9
Parsnip	*Pastinaca sativa*			
Pate (patete)	*Schefflera digitata*	4		
Patotara	*Leucopogon fraseri*	4		
Pea	*Pisum sativum*	6		
Peach	*Prunus persica*	7	7	7
Pear	*Pyrus communis*	7	7	7
Pearlwort, prostrate	*Sagina procumbens*	1		
Pennyroyal	*Mentha pulegium*	7,8		7,8
Pentstemon	*Pentstemon* spp.	6,7		7
Petunia	*Petunia* sp.			
Phlox	*Phlox* sp.	6		
Pigeonwood	*Hedycarya arborea*			
Pimpernel, scarlet	*Anagallis arvensis*		6	
Piripiri (bidibid)	*Acaena sanguisorbae*		4	
Piripiri, Australian	*A. ovina*		+	
Plantain, greater	*Plantago major*		2	
Plantain, narrow-leaved	*P. lanceolata*		7,9	7,9
Pohuehue	*Muehlenbeckia complexa*		+	
Polygala	*Polygala* sp	1		
Poplar	*Populus nigra*		6,9	
Poppy, field	*Papaver rhoeas*	7	7,9	7,9
Poppy, iceland	*P. nudicaule*	7		7
Poppy, opium	*P. somniferum*	7		7
Poppy, oriental	*P. orientale*	7	9	7,9
Poroporo	*Solanum aviculare*		+	
Potato	*S. tuberosum*		+	
Pratia	*Pratia angulata*	4		
Pukatea	*Laurelia novae-zealandiae*			
Purpletop	*Verbena bonariensis*	6,7		7
Ragwort	*Senecio jacobaea*	6		
Rangiora	*Brachyglottis repanda*	3		
Rata	*Metrosideros diffusa*	+		
Rauraki, sow-thistle	*Sonchus oleraceus*	7	7	7
Rewarewa	*Knightia excelsa*	3,8	3	8
Rhododendron	*Rhododendron* sp.	6		
Rose, briar (dog)	*Rosa canina*		1	
Rose, climbing	*R. multiflora*		7	
Rose, sweet briar	*R. eglanteria*		7	
Rose, tea	*R. indica*		7	
Rosemary	*Rosmarinus officinalis*	7		7
Salpiglossus	*Salpiglossus* sp.			
Salvia	*Salvia* sp.	7	9	7,9
Satureia	*Satureja vulgaris*	6		
Scarlet pimpernel	*Anagallis arvensis*		6	
Sedum	*Sedum* sp.	6		7
Self-heal	*Prunella vulgaris*	6		
Snowberry	*Gaultheria antipoda*	4		
Sorrel, sheep	*Rumex acetosella*		9	9
Sow-thistle	*Sonchus asper*	6		
Speedwell, field	*Veronica arvensis*	1,7	9	7,9
Spiraea	*Spiraea* sp.	7	7	7
Strathmore weed	*Pimelea prostrata*	4		
Strawberry, wild	*Fragaria vesca*	7	7	7
Supplejack	*Rhipogonum scandens*			
Sumac (poison ivy)	*Rhus* sp.	7		
Sweet William	*Dianthus barbatus*	6		
Tarweed	*Odontites viscosa*			
Tauhinu	*Pomaderris phylicaefolia*			
Tawa	*Beilschmiedia tawa*			
Thistle, Californian	*Cirsium arvense*	7	7	7
Thistle, Scotch (spear)	*C. lancolatum*	7	7,9	7,9
Thrift	*Armeria* sp.	6		
Titoki	*Alectryon excelsum*	8		8
Trefoil, haresfoot	*Trifolium arvense*	6		
Trefoil, hop	*T. campestre*	6		
Turnip, wild	*Brassica campestris*	7	9	7,9

Common Name.	Botanical Name.	Nectar Flowers.	Pollen Flowers	Worked by Bees.
Tutu	*Coriaria arborea*		4	
Viburnum	*Viburnum* sp.		7,9	7,9
Guelder rose	*V. opulus*	6		
Viper's bugloss	*Echium* sp.	7,8		7,8
Virginia creeper	*Parthenocissus quinquefolia*	7	7	7
Viscaria	*Lychnis* sp.	1		7
Wallflower	*Cheiranthus cheiri*	6		
Walnut	*Juglans* sp.		7,9	9
Wattle	*Acacia longifolia*			
Willow, pussy	*Salix caprea*	7	7,9	7,9
Wireweed	*Polygonum aviculare*		+	
Yarrow	*Achillea millefolium*	7		7
	Hypericum japonicum			
	Stellaria parviflora	7		7

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THE AMMONIA AND NITRATE CONTENT OF GLASSHOUSE TOMATO SOIL UNDER DIFFERENT TREATMENTS

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Summary

The effect of sterilization with steam, chloropicrin, D-D and gammexane on the ammonia and nitrate content of the soil was examined These methods of sterilization differed in their effect on nitrification in the soil.

All methods of sterilization brought about increases in yield and growth. Gammexane at the rate used was the least effective. Differences in available nitrogen between sterilized and unsterilized plots did not seem to be sufficient to account for the differences in yield and growth. High ammonia nitrogen present in the early stages of plant growth in certain sterilized plots did not appear to affect the yield of fruit. Heavy dressings of cocoa husks increased growth and yield on unsterilized soil and increased the inorganic nitrogen level of the soil.

INTRODUCTION

IN studying the effect of soil sterilization on the growth of tomato plants and on the yield and quality of the fruit, determinations were made of the nitrogen present in the soil as ammonia and nitrate in certain glasshouse plots at different times during the growing season. Information was obtained on changes taking place in the available nitrogen content of the soil with different methods of sterilization and with certain modification of the standard fertilizer treatment.

Results are given in Tables I and II for six plots with different treatments in Glasshouse A sampled at intervals from August to December, and Table III gives figures for a number of plots in Glasshouse B where four methods of soil-sterilization were being compared.

TREATMENTS IN GLASSHOUSE A

The standard fertilizer treatment consisted of :—

(a) the applications of 4 oz. superphosphate and 2 oz. sulphate of potash per sq. yd. at the time of digging during the winter.

(b) the use prior to planting of a mixture supplying 2 oz. superphosphate 1 oz. sulphate of potash, ¾ oz. dried blood and ¾ oz. sulphate of ammonia per sq. yd.

Half this quantity was broadcast and forked in over the whole plot, and the other half was raked in along the trenches. The fertilizer was applied on 8th August and the plants were set out in the trenches on 21st August. A top-dressing of the fertilizer mixture at the previous rate, i.e., 4½ oz. per sq. yd., calculated on the total area of the plot, was applied along the rows on 5th November.

Where cocoa husks were used, they were spread over the whole plot and dug into the top 6-8 in. after sterilization.

Steam sterilization was carried out to a depth of 12-14 in. The steam was applied at approximately 100 lb. pressure for about 5 minutes, i.e., until it came freely through to the surface. The date of steaming was 9th June. Chloropicrin was applied on 13th June. It was injected into the soil at a depth of 6 in. at the rate of approximately 6 ml. per sq. ft.

The experimental plots were treated as follows :—

PLOT A6 Steam-sterilized, standard fertilizer.

PLOT A8 Steam-sterilized, standard fertilizer except that the nitrogen was supplied as dried blood, cocoa husks (1947), 10 tons dry matter per acre.

PLOT A12 Chloropicrin-sterilized, standard fertilizer, cocoa husks (1947), 10 tons dry matter per acre.

PLOT A19 Unsterilized, standard fertilizer, cocoa husks (1946 and 1947), 10 tons dry matter per acre each year.

PLOT A20 Unsterilized, standard fertilizer.

PLOT A22 Steam-sterilized, standard fertilizer, cocoa husks (1947), 10 tons dry matter per acre.

The soil in this glasshouse and in glasshouse B had a pH of approximately 7 throughout.

SAMPLING OF THE PLOTS

Soil samples were taken with a half-inch soil borer in the trenches between the plants as near the line of the row as possible and each sample consisted of 12 to 15 cores to a depth of 9 in. Except where specified the time of sampling was between 8.30 a.m. and 10 a.m. and the nitrogen determinations were carried out the same day.

It may be pointed out that under glasshouse conditions it is more difficult to obtain an even distribution of applied nitrogen than in plot experiments. For this reason small differences in ammonia and nitrate content between samples may have little significance. In considering the ratio of ammonia nitrogen to nitrate nitrogen in the soil, the possibility that the plant may absorb one form of nitrogen more rapidly

TABLE 1. GLASSHOUSE A. SEASON 1947-48
Ammonia and Nitrate Nitrogen in Glasshouse Tomato Plots in p p.m. of dry soil

Plot Nos.	19 Aug.	1 Sep.	15 Sep.	30 Sep.	13 Oct.	28 Oct.	3 Nov.		17 Nov.	1 Dec
PLOT A6. Steam-sterilized								TOP-DRESSED 5th NOVEMBER		
Ammonia nitrogen ...	89	95	46	25	7	5	7		57	7
Nitrate	7	31	52	51	51	39	32		22	27
Ammonia and nitrate nitrogen	96	126	98	76	58	44	39		79	34
Yield of fruit = 7.1 lb										
PLOT A8. Steam-sterilized, Organic nitrogen, cocoa husks 1947										
Ammonia nitrogen ...	41	85	26	16	4	25	9		37	12
Nitrate	7	43	88	64	60	99	42		54	81
Ammonia and Nitrate nitrogen	48	128	114	80	64	124	51		91	93
Yield of fruit = 6.7 lb										
PLOT A20. Unsterilized										
Ammonia nitrogen ...	63	28	8	8	1	2	2		21	3
Nitrate	22	86	110	73	38	90	52		74	56
Ammonia and nitrate nitrogen	85	114	118	81	39	92	54		95	59
Yield of fruit = 3.8 lb.										
	21 Aug	3 Sep.	17 Sep	1 Oct	15 Oct	30 Oct.	5 Nov.		18 Nov.	2 Dec.
PLOT A12 Chloropicrin, cocoa husks 1947								TOP-DRESSED 5th NOVEMBER		
Ammonia nitrogen ...	119	122	99	97	85	45	41		78	39
Nitrate	2	4	6	8	13	31	32		52	55
Ammonia and nitrate nitrogen	121	126	105	105	98	76	73		130	94
Yield of fruit = 7.5 lb										
PLOT A19. Unsterilized, cocoa husks 1946 and 1947										
Ammonia nitrogen ...	54	13	13	6	5	2	4		28	8
Nitrate	54	115	133	92	112	109	101		89	111
Ammonia and nitrate nitrogen	108	128	146	98	117	111	105		117	119
Yield of fruit = 5.5 lb.										
PLOT A22. Steam sterilized, cocoa husks 1947										
Ammonia nitrogen ...	81	54	19	11	8	10	8		55	16
Nitrate	14	55	68	53	51	44	35		47	54
Ammonia and nitrate nitrogen	95	109	87	64	59	54	43		102	70
Yield of fruit = 7.7 lb.										

NOTE: Yields expressed as average per plant for the whole season.

than another must be borne in mind. However, from the results in Table I certain general conclusions may be drawn.

1. The applied nitrogen was converted rapidly into nitrates in the unsterilized soil, so that by the middle of September most of the available nitrogen in Plots A19 and A20 was in the nitrate form.

2. Steam delayed the nitrifying process as may be seen from the ammonia figures for 1st September.

3. Chloropicrin at the rate used in Plot 12, i.e., 6 ml. per sq. ft. was more effective than steam in delaying nitrification. By early November when the top-dressing was applied, there was still more nitrogen present as ammonia than as nitrate in this plot.

4. Dried blood applied to steam-sterilized soil rapidly gave rise to ammonia (Plot 8), showing that organisms capable of bringing about the decomposition of organic nitrogen compounds to the ammonia stage were active when the fertilizer was applied.

5. Even after the nitrifying power of the soil appeared to be completely restored, the nitrogen of the top-dressing (applied on 5th November) was changed to nitrate more slowly where the soil had previously been sterilized than on unsterilized plots. Thus on 17th November, the sterilized plots gave ammonia figures from 37 to 78, averaging 57 p.p.m. of nitrogen while the amounts found in the unsterilized plots were 21 and 28 p.p.m.

6. From a consideration of the figures for total ammonia plus nitrate nitrogen, it seems improbable that the increase in available nitrogen which results from soil-sterilization provides an adequate explanation of the large increases in yield obtained where steam or chloropicrin had been used. In the unsterilized plot A20 where the yield averaged only 3.8 lb. per plant compared with 7.1 lb. for a steamed plot with the same fertilizer treatment (A6), the soil in the trenches was well supplied with available nitrogen throughout the period of the experiment. The most consistently high level of available nitrogen was found in the unsterilized plot A19 where cocoa husks at the rate of 20 tons per acre in two years and equivalent to approximately 600 lb. of nitrogen (N) per acre each year had been applied. This plot gave a yield of 5.5 lb. per plant compared with 3.8 lb. for the unsterilized plot without husks (A20); but the improvement in both growth and yield was not as great as that produced by steam sterilization (A6). Cocoa husks are a rich source not only of nitrogen but of other plant foods. The beneficial effect of soil-sterilization seems to be due more to the destruction of root diseases and the consequent stimulation of fine root growth than to any other factor in the Nelson tomato soils.

On 17th November the 9 to 15 in. depth in three plots was analysed. Table II gives a comparison between the 0-9 in. and 9-15 in. depths at this time.

TABLE II. AMMONIA AND NITRATE NITROGEN CONTENTS OF THE 0-9 IN. AND 9-15 IN. DEPTHS OF SOIL.

Depth.	Ammonium N p.p.m.			Nitrate N p.p.m.		
	Plot A6	Plot A8.	Plot A20.	Plot A6.	Plot A8.	Plot A20
0 - 9 in	57	37	21	22	54	74
9 - 15 in.	14	13	9	5	9	9

It appears that on this type of soil under the fertilizer system used, the concentration of ammonia and nitrate nitrogen in the soil below the top nine inches is small.

TREATMENTS IN GLASSHOUSE B

All plots tested for available nitrogen in Glasshouse B had been given the standard fertilizer treatment. With the exception of plots B3 and B5 they had received 10 tons per acre of cocoa husks on the dry matter basis in 1946 and 10 tons in 1947. Plot B3 had no husks in 1946 and a heavy dressing of 30 tons per acre in 1947. Plot B5 had no husks in 1946 and 10 tons per acre in 1947.

STERILIZATION

Steam :

Steaming was carried out as in Glasshouse A. The date of steam treatment was 6th June.

D-D :

D-D was injected into the soil at a depth of 6 in. at the rate of 5 ml per sq. ft. on 18th June.

Chloropicrin :

Chloropicrin was injected into the soil at a depth of 6 in. at the rate ot 4 ml per sq. ft. on 16th June.

Gammexane :

Gammexane was used at the rate of 10 lb. per acre containing 10 to 12 per cent. active agent. Half the quantity was dug into a 6 in. depth, a quarter broadcast and forked and a quarter left broadcast on the surface. It was applied on 16th June.

TABLE III. GLASSHOUSE B. SEASON 1947-48

Effect of Different Methods of Sterilization on the Formation of Nitrate in the Soil

Results expressed as nitrogen in p.p.m of the dry soil

	Plot B2	Plot B3	Plot B4	Plot B5	Plot B6	Plot B7	Plot B8
	ST	U 30H	D-D	CL	U	GA	ST
SAMPLED 13th OCTOBER*							
Ammonia nitrogen ...			83	15		2	6
Nitrate nitrogen			13	75		51	89
Ammonia and nitrate nitrogen			96	90		53	95
SAMPLED 21st OCTOBER							
Ammonia nitrogen ...		17		34	2	2	
Nitrate		149		73	61	73	
Ammonia and nitrate nitrogen		166		107	63	75	
	TOP	DRESS	ED 4th	NOVE	MBER		
SAMPLED 3rd AND 4th DECEMBER							
Ammonia Nitrogen ...	13	23	40	28	9	17	10
Nitrate	63	119	57	63	62	77	77
Ammonia and nitrate nitrogen	76	142	97	91	71	94	87
SAMPLED 16th AND 17th DECEMBER							
Ammonia nitrogen ...	11	10	20	15	6	4	
Nitrate	40	93	50	52	39	34	
Ammonia and nitrate nitrogen	51	103	70	67	45	38	
Average yield of fruit in lb. per plant	8.5	7.1	7.4	8.3	4.9	6.5	7.7

ST = Steam
CL = Chloropicrin
U = Unsterilized
30H = 30 tons cocoa husks per acre
GA = Gammexane

* Samples on 13th October were taken in the late afternoon and analyses carried out next morning.

The results show that the effect on the nitrifying power of the soil was most noticeable with D-D. Nitrification was less retarded with chloropicrin and least with steam and gammexane. Observation showed that where sterilizing agents retarded the change from ammonia to nitrate so that the main supply of inorganic nitrogen to the plant was in the form of ammonia for a long period, the plants tended to be less vigorous than on other sterilized plots. Thus the slowest-growing plants on sterilized soil during October and November were found with D-D (plot 4) in glasshouse B and with chloropicrin (plot 12) in glasshouse A. The final yield of fruit, however, was not adversely affected. Whether the tomato plants grew more vigorously with nitrate than with ammonia or whether the retarded growth in plots B4 and A12 was due to toxic residues left in the soil after sterilization with chloropicrin or D-D has not been determined. It was noticed that a garlic-like odour was given off from plot B4 when sterilized by steam a year after the D-D treatment.

Under the conditions of the experiment, steam and chloropicrin were the most effective and gammexane the least effective sterilizing agents in increasing fruit production (Table II). As in glasshouse A, an improvement in growth and yield was associated with the use of cocoa husks on unsterilized soil. Plot B3 where 30 tons of husks had been applied in the 1947-48 season, instead of the standard 10 tons, gave a yield of 7.1 lb. per plant compared with 4.9 lb. for the unsterilized plot on the standard treatment, and plant growth was more vigorous. The highest figures for available nitrogen were for this plot, but the effect of the heavy dressing of cocoa husks on growth and yield was not as marked as that produced by sterilization with steam or chloropicrin.

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THE EFFECT OF STEAM AND CHLOROPICRIN TREATMENT ON THE AMMONIA AND NITRATE NITROGEN CONTENT OF A NELSON TOMATO SOIL

By E. B. KIDSON, Cawthron Institute, Nelson

(Received for publication, 29th September, 1948)

Summary

As part of an investigation into the effect of soil-sterilization on the growth and yield of tomato plants, pot experiments were carried out over a period of seven months to determine the effect of chloropicrin and steam treatments on the ammonia and nitrate content of a Nelson tomato soil, with and without fertilizer. It was found that steam sterilization was more effective than the chloropicrin treatment in raising the level of available nitrogen in the soil. Chloropicrin at the rate used (4 ml. to 26 lb. of soil) was shown to delay nitrification some two months longer than steam.

FOLLOWING on the work carried out by Russell and others at the Rothamsted Experimental Station (1), the use of steam for the partial sterilization of tomato soils was introduced in the Nelson district some 20 years ago, and has now become a standard glasshouse practice, repeated tests at the Cawthron Institute having shown the considerable increase in growth and yield associated with steam sterilization.

In recent years chloropicrin has been used with great success in the U.S.A. for the sterilization of intensely-cropped soils. Experiments to determine the effectiveness of chloropicrin-treatment of the soil in Nelson tomato houses have given satisfactory results, comparable to those obtained with steam. The use of chloropicrin has resulted in a great improvement in growth and an increase of about 3 lb. per plant in yield.

The exact role of steam and chloropicrin in causing such a marked increase in growth and yield is not known. The elimination of soil-borne diseases must play an important part. Chemical and physical changes brought about by the action of the sterilizing agent and by the alteration in the soil population after sterilization have not yet been fully elucidated. Russell and many later workers (1) have demonstrated that sterilization has an important effect on the nitrogen supply of the plant. Partial sterilizarion of the soil with steam and certain chemicals is followed by a rise in ammonia nitrogen in the soil. Nitrification however is inhibited for a period the length of which depends on the sterilizing agent. During this period ammonia formed in the soil or added as fertilizer remains as ammonia and is not changed to nitrate as in unsterilized soils.

It was considered that information of the amount of nitrogen present as ammonia and nitrate in Nelson tomato soil under different methods of treatment would be of value in a study of the effect of soil sterilization on the yield and growth of tomato plants. The present paper gives an account of pot experiments to determine the effect of steam-sterilization and chloropicrin-treatment on the ammonia and nitrate nitrogen in a soil of the type on which most of the Nelson tomato houses are located. The soil is characterized by a fairly heavy texture, a pH of about 7 and a high content of exchangeable calcium and magnesium. The phosphate and potassium tend to be low though fertilizer treatment has raised the phosphate content to a high figure in places. In most parts the soil is low in organic matter. The sample of soil used for the pot experiments contained 17.3 m.e. per cent. of calcium, 7.6 m.e. per cent. of magnesium and less than 0.1 m.e. per cent. of potassium. The pH was 7.1 and the organic carbon and nitrogen were 1.65 and 0.17 per cent. respectively. The amount of P_2O_5 soluble in 1 per cent. citric acid was 0.14 per cent.

EXPERIMENTAL

Glazed earthenware pots holding about 26 lb. of soil were used for the experiment. They were divided into two series, (a) without fertilizer and (b) with added fertilizer. Both series contained four pots of unsterilized soil, four of steam-sterilized soil and four of chloropicrin-treated soil. The steamed soil was sterilized in bulk in the usual way on 6th June, 1947. Where chloropicrin was used it was applied in each plot to a depth of 6 in. at the rate of 4 ml. per plot of 26 lb. of soil. The chloropicrin treatment was carried out on 13th June and the pots were then left covered for several days.

In the fertilizer series the fertilizer was thoroughly mixed with the soil. The date of application was 13th June, (before the chloropicrin treatment) and the rate was 2 oz. per pot of a mixture containing superphosphate eight parts, sulphate of potash four parts, sulphate of ammonia one part and dried blood one part, supplying approximately 115 p.p.m. of nitrogen.

Late in June all pots were brought to a moisture content of 60 per cent. saturation and the soil mixed. Water was given periodically throughout the experiment to maintain a weight equivalent to 60 per cent. saturation, tap water being used throughout. The pots were kept free of weeds.

Sampling of the 0-6 in. depth for the estimation of ammonia and nitrate nitrogen was commenced about a month after the application of the fertilizer and continued at intervals until the middle of January. The samples were all taken between 8.30 a.m. amd 9.30 a.m. and the analyses carried out immediately. Three cores were taken from each pot to a depth of 6 in. with a cylindrical soil borer, which was a half inch in diameter. The soil not used for analysis was replaced in the pots.

METHODS OF ANALYSIS

The nitrate nitrogen was estimated by the phenoldisulphonic acid method and the ammonia by extraction with neutral 10 per cent. KCl and distillation with magnesia.

RESULTS

The results obtained are given in Table I and are shown diagrammetically in Figs. 1, 2 and 3.

Table I. Effect of Steam and Chloropicrin Sterilization on the Ammonia and Nitrate Nitrogen Content of a Nelson Soil With and Without Fertilizer

I. No Fertilizer Series. Parts Per Million of Nitrogen on Dry Soil

Date of Sampling.	10/7/47	23/7	5/8	25/8	8/9	22/9	6/10	20/10	11/11	24/11	8/12	30/12	12/1/48
(a) *Ammonia Nitrogen*													
Unsterilized	4	3	2	1	2	1	1	2	3	5	4	2	2
Steam-sterilized	13	18	10	5	4	2	1	3	3	10	4	3	3
Chloropicrin-sterilized	8	14	12	14	15	12	9	8	4	11	5	3	3
(b) *Nitrate Nitrogen*													
Unsterilized	9	8	9	7	8	7	7	7	15	15	11	11	15
Steam-sterilized	7	8	17	23	29	26	26	28	44	36	35	32	35
Chloropicrin-sterilized	7	9	7	6	6	5	7	16	27	26	22	18	28

II. Fertilizer Series. Parts Per Million of Nitrogen on Dry Soil

Date of Sampling.	14/7/47	25/7	7/8	27/8	10/9	24/9	8/10	22/10	13/11	25/11	9/12	29/12	13/1/48
(a) *Ammonia Nitrogen*													
Unsterilized	25	4	3	2	2	2	1	2	6	4	4	1	4
Steam-sterilized	91	93	79	22	7	5	1	3	4	2	3	4	4
Chloropicrin-sterilized	66	67	69	75	82	72	50	41	13	7	4	4	4
(b) *Nitrate Nitrogen*													
Unsterilized	62	67	69	92	94	61	74	60	64	61	59	58	55
Steam-sterilized	7	10	16	80	95	80	79	87	74	65	67	64	62
Chloropicrin-sterilized	9	8	7	7	5	9	31	48	62	61	61	71	69

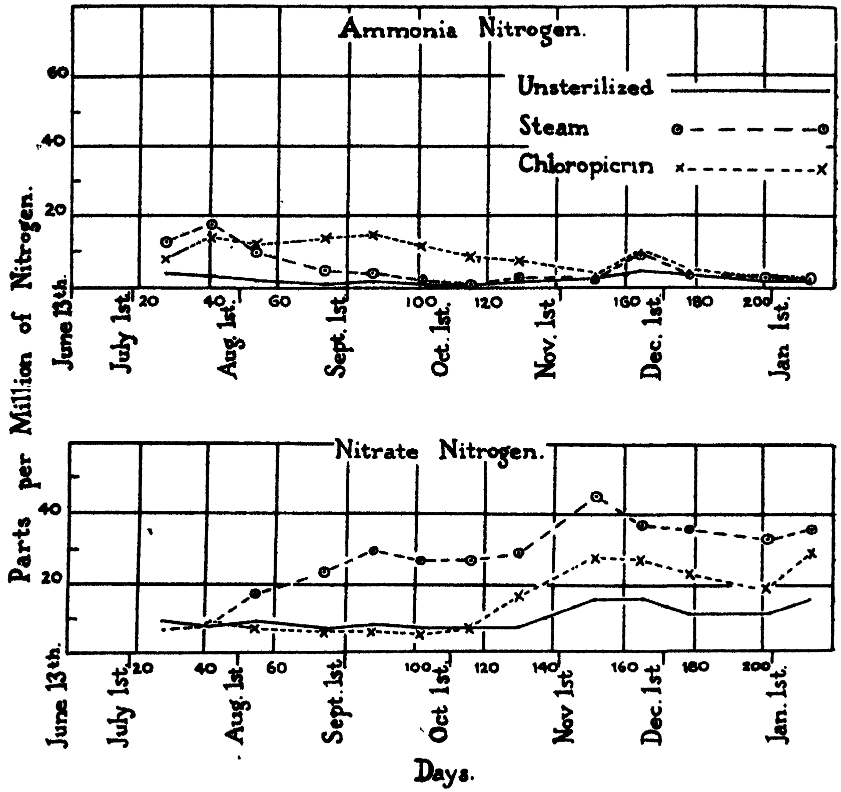

FIG. 1

NO FERTILIZER SERIES

AMMONIA NITROGEN.

In the unsterilized soil the ammonia nitrogen remained very low throughout the experiment, never exceeding 5 p.p.m. Steam treatment caused a slight rise to 18 p.p.m. in July, followed by a drop to figures comparable with those of the unsterilized soil. Chloropicrin treatment produced a similar rise, the increased ammonia content being maintained some two months longer.

NITRATE NITROGEN.

Steaming brought about an appreciable increase in the nitrate nitrogen content over that of the unsterilized soil, presumably owing to increase in bacterial activity. Thus on 8th September, the unsterilized soil contained 8 p.p.m. and the steam-sterilized soil 29 p.p.m. and on 11th November the corresponding figures were 15 p.p.m. and 44 p.p.m. A similar but smaller effect was produced in the chloropicrin-treated soil, delayed about two months owing to the slow re-establishment of nitrification with the chloropicrin treatment. The maximum nitrate nitrogen figures reached in the unsterilized, steam-sterilized and chloropicrin-treated soils were 15 p.p.m., 44 p.p.m. and 28 p.p.m. respectively. For all treatments a rise in nitrate occurred in the October-November period, followed by a slight drop in December.

TOTAL NITRATE AND AMMONIA NITROGEN.

The effect of steam and chloropicrin in raising the level of ammonia plus nitrate in the soil can be clearly seen in the no-fertilizer series in Fig. 3. The increase brought about by both these methods was apparent from the time of the first sampling on 10th July, i.e. 34 days after steaming and 27 days after the application of chloropicrin. The chloropicrin treatment was less effective in this respect than the steaming. Throughout the experiment available nitrogen formed as a result of the chloropicrin treatment was about half that produced by the steam sterilization. Some of this increase may be due to the decomposition of chloropicrin residues in the soil. The amount applied contained nitrogen equivalent to about 50 p.p.m. but considering the volatility of chloropicrin, it is expected that most of this 50 p.p.m. was lost by evaporation.

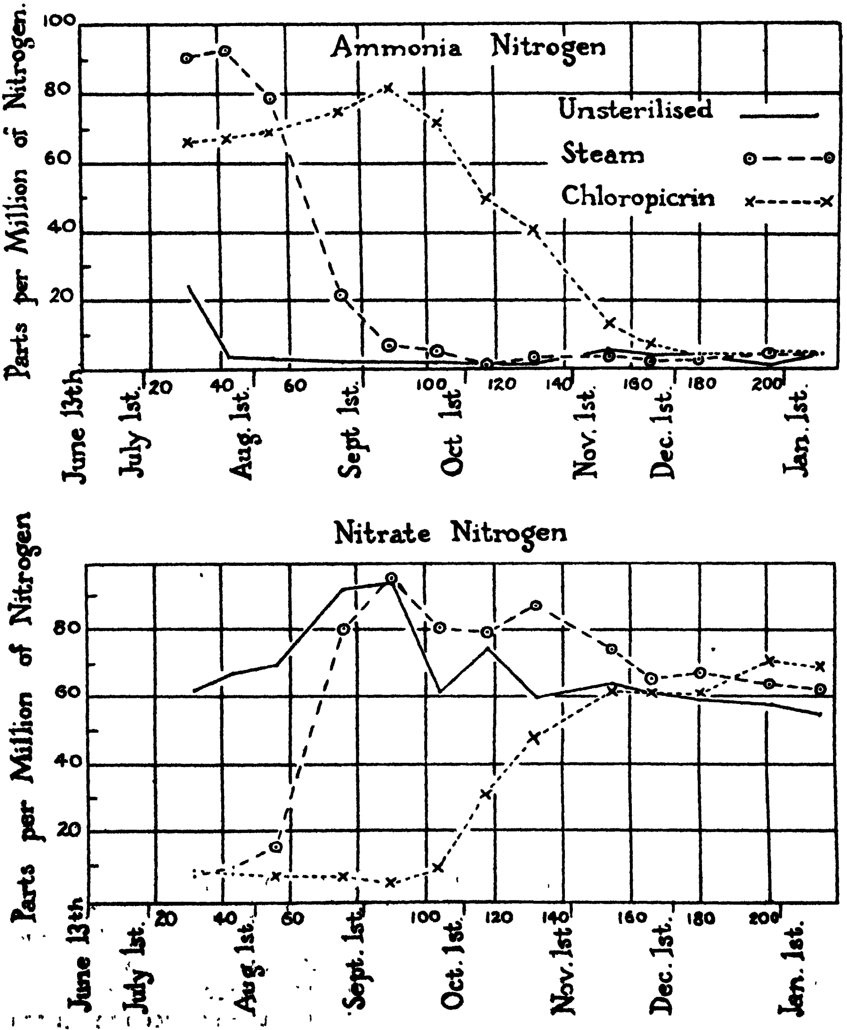

FIG. 2

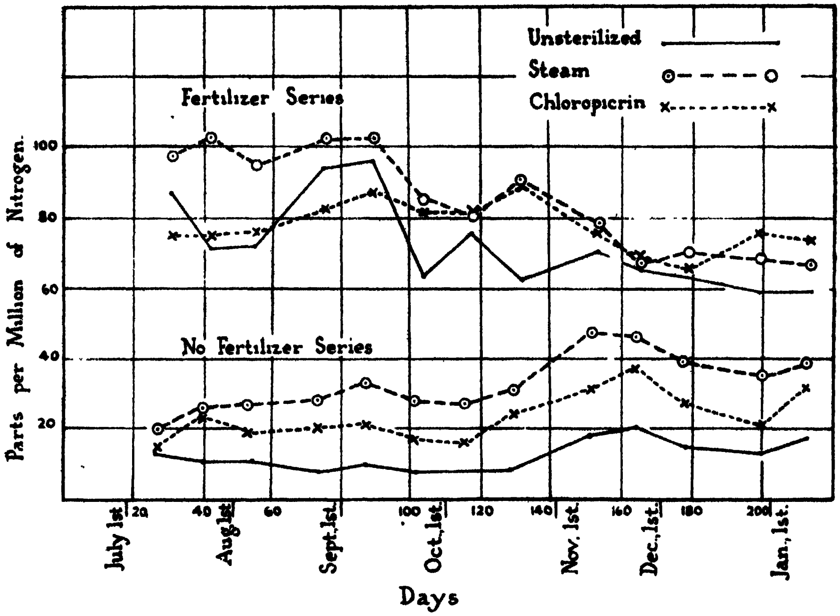

FIG. 3

FERTILIZER SERIES

The fertilizer used supplied nitrogen equivalent to approximately 115 p.p.m., 70 p.p.m. in the form of sulphate of ammonia and 45 p.p.m. as dried blood.

AMMONIA NITROGEN.

The fertilizer was applied on 13th June and by 14th July, at the time of the first sampling, nitrification was almost complete in the unsterilized pots. Only 25 p.p.m. of ammonia nitrogen was found, and this dropped rapidly to a very low figure. For the steamed pots the first figures were high (91 and 93 p.p.m.) but in six to seven weeks from the date of the application of the fertilizer, a rapid decrease had begun, and the ammonia nitrogen dropped to, and remained at that of the unsterilized pots. Allowance being made for the seven days between the steam and chloropicrin treatments, it will be seen that the chloropicrin delayed nitrification considerably longer, a result in agreement with that obtained with the unfertilized soil. Thus the ammonia nitrogen fell to less than 10 p.p.m. in about 40 days after the fertilizer application where no sterilization was given, in 80 to 90 days with steam-sterilization and in 160 days with the chloropicrin treatment. It was noticeable that even when the normal nitrifying power of the soil appeared to be restored, nitrification was slower in the chloropicrin-treated soil than the steamed soil. There are some indications also that chloropicrin may have slowed down the production of ammonia from the dried blood. The initial ammonia nitrogen figure on 14th July was 66 p.p.m. which showed a slow rise to 82 p.p.m. over the next 58 days, presumably owing to the formation of ammonia from dried blood. However, the sterilization of the fertilizer with the soil in the chloropicrin treatment may have had some effect on the speed of decomposition of dried blood in the early stages.

NITRATE NITROGEN.

By 14th July the unsterilized soil had a nitrate nitrogen content of 62 p.p.m. which rose to a maximum about the beginning of September. In the steam- and chloropicrin-sterilized soils the nitrate nitrogen content at the first sampling was 7 and 9 p.p.m. respectively, but with the steam-treatment analyses showed a rapid formation of nitrate commencing about 20 days later, and corresponding to the fall in ammonia content. In the chloropicrin-treated pots the formation of nitrate from the ammonia of the fertilizer was delayed approximately a further 50 days and was slower than where steam had been used.

TOTAL NITRATE AND AMMONIA NITROGEN.

For the fertilizer series (Fig. 3) the steamed soil had a higher content of available nitrogen throughout the experiment than did the unsterilized soil. In the chloropicrin-treated soil the available nitrogen did not rise above that of the unsterilized soil until late September, after which steam and chloropicrin gave almost identical values. It is of interest to note that in the fertilizer series the nitrogen present as nitrate and ammonia did not at any time reach 115 p.p.m., the amount actually supplied by the fertilizer. Also in both sterilized and unsterilized pots of this series there was a slow decrease after the middle of September. Analysis showed that this decrease could not be explained by leaching of nitrate into the soil below the usual sampling depth. It is presumed to be due to the removal of available nitrogen by algae and other micro-organisms.

CONCLUSION

It appears that chloropicrin at the rate used, (somewhat higher than the local commercial rate) is not as effective as steam in raising the level of easily available nitrogen in the soil. Thus, in the unfertilized soil, steam-sterilization brought about an increase of some 20 p.p.m. of nitrogen and chloropicrin-treatment approximately 10 p.p.m., some of which may have been due to the decomposition of chloropicrin residues in the soil.

The nitrifying power of the soil was more affected by the chloropicrin-treatment than by the steam-sterilization. Where chloropicrin had been used, ammonia formed in the soil as a result of sterilization began to change to nitrate about two months later than in steamed soil. A similar effect was observed in the nitrification of the ammonia from applied nitrogenous fertilizers. It is to be expected that different results would be obtained with different quantities of chloropicrin.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance given by Mr. J. Robinson and Mr. E. Marshall in carrying out this work.

REFERENCE

(1) RUSSELL, E. J and HUTCHINSON, H. B. (1909): *J. Agr. Sci.*, 3, 3.

CHEMICAL CONTROL OF *OXYCANUS CERVINATUS* WALKER

IV. EXPERIMENTS IN 1947 SEASON

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(*Received for publication, 31st December, 1947*)

Summary

No equally cheap, efficient and readily available substitute for bran in the Paris Green-bran bait has been discovered. Gristed oats gave partial control but was more costly and presented problems in mixing and spreading. The addition of 2½ lb. of wheat germ to 50 lb. of sawdust or chaff improved their attractiveness as carriers. D.D.T. in dosages of 0.6 lb. of the para para isomer per acre gave good control, both as sprays and as dusts. Gammexane dusts, at an equivalent dosage of the gamma isomer per acre, gave equally good control of *Oxycanus cervinatus*.

THE poisoned bran bait which was developed for the control of subterranean grass caterpillar in pasture by Dumbleton and Dick (1, 2, 3) has proved to be effective and reasonably cheap, especially on pastures devoted to grass and clover seed production. Supplies of bran are, however, always limited and at present are controlled. Farmers have been faced with difficulty in securing bran and threatened with prohibition of the use of bran in poison baits for insect control. For this reason an experiment designed to test the effectiveness of possible alternative materials was laid down in the autumn of 1947. The opportunity was also taken to test out the newer organic insecticides applied as dusts and sprays.

LOCATION, LAYOUT, TREATMENTS AND SAMPLING METHOD

The experimental area was a run-out pasture on the Bruce Estate at Seafield, near Ashburton. The experiments were laid out as randomized blocks with three replications of each treatment. The individual plots were ½ chain by ¼ chain (1/80 acre). In Experiment 1 there were no buffer areas between plots or between blocks. In Experiments 2 and 3 there were ¼ chain buffer areas between blocks, but none between plots. The treatments were applied, in Experiments 1 and 2 on 15th March, and in Experiment 3 on 28th March and 2nd April.

Poison baits were broadcast by hand, dusts were applied with a hand dust gun of the plunger type and sprays with a knapsack sprayer.

The details of the experiments are as follows :—

Experiment 1. Poison Baits and D.D.T. Spray (*Quantities per Acre*)

Treatment No. 1 Paris Green 2 lb. 1 oz.; bran 50 lb.
2 Wettable D.D.T. (20 per cent. p.p.i.) 2½ lb.; water 100 gal.
3 As_2O_3, 2 lb. 1 oz.; bran 50 lb.
4 Paris Green 2 lb. 1 oz.; gristed oats 50 lb.
5 Paris Green 2 lb. 1 oz.; sawdust 50 lb.
6 Paris Green 2 lb. 1 oz.; sawdust 50 lb.; white flour 2½ lb.

7 Paris Green 2 lb. 1 oz.; sawdust 50 lb.; 80 per cent. flour 2½ lb.
8 Paris Green 2 lb. 1 oz.; sawdust 50 lb.; high B_1 flour 2½ lb.
9 Paris Green 2 lb. 1 oz.; sawdust 50 lb.; wheat germ 2½ lb.
10 Paris Green 2 lb. 1 oz.; chaff 50 lb.
11 Paris Green 2 lb. 1 oz.; chaff 50 lb.; white flour 2½ lb.
12 Paris Green 2 lb. 1 oz.; chaff 50 lb.; 80 per cent. flour 2½ lb.
13 Paris Green 2 lb. 1 oz.; chaff 50 lb.; high B_1 flour 2½ lb.
14 Paris Green 2 lb. 1 oz.; chaff 50 lb.; wheat germ 2½ lb.
15 Paris Green 2 lb. 1 oz.; coarse gristed oats 50 lb
16 Control.

Experiment 2. D.D.T. Dusts (Quantities per Acre)—

Treatment No. 1 Paris Green 2 lb. 1 oz.; bran 50 lb.
2 Control
3 D.D.T. 2 per cent. (p.p.i.) dust at 120 lb.
4 D.D.T. 2 per cent. (p.p.i.) dust at 60 lb.
5 D.D.T. 2 per cent. (p.p.i.) dust at 30 lb.
6 D.D.T. 2 per cent. (p.p.i.) dust at 15 lb.

Experiment 3. Gammexane Dusts (Quantities per Acre)—

Treatment No. 1 Paris Green 2 lb. 1 oz.; bran 50 lb.
2 Control
3 Gammexane 4 per cent. dust (0.5 per cent. g.i.) 120 lb.
4 Gammexane 4 per cent. dust (0.5 per cent. g.i.) 60 lb.
5 Gammexane 4 per cent. dust (0.5 per cent. g.i.) 30 lb.
6 Gammexane 4 per cent. dust (0.5 per cent. g.i.) 15 lb.

The plots were sampled in mid-May, two months after treatment. Ten samples, each one foot by one foot, were taken at random within each plot and the number of living *Oxycanus* larvae and *Odontria zealandica* larvae were recorded for each sample. Before sampling was commenced the plots were graded for vegetation cover separately by two observers. An arbitrary visual grading was used, " 0 " being the cover on the worst of the untreated plots and " 10 " being the cover on the best of the plots treated with the Paris Green and bran bait (Experiment 1, Treatment No. 1).

EXPERIMENTAL RESULTS

The data relating to surviving *Oxycanus* caterpillars which were subjected to an analysis of variance reveals that there are significant treatment effects. The differences in treatment means required for significance are 3.41 on the 5 per cent. level and 4.59 on the 1 per cent level.

TABLE I. EXPERIMENT 1. POISON BAITS AND D.D.T. SPRAY

Treatment No.	No. of living *Oxycanus* caterpillars per sq. ft.			Treatment Mean	No. of living *O. zealandica* larvae per sq. ft.			Treatment Mean	Grading of Vegetation cover after treatment.			Treatment Mean
	Block. A	B	C		Block. A	B	C		Block. A	B	C	
1	1.2	1.1	2.8	1.66	11.1	37.4	39.6	29.36	9	8	8	8
2	6.1	3.1	2.7	3.96	24.3	44.3	18.4	29.0	8	8	10	8
3	11.5	7.0	11.5	10.0	30.0	20.0	19.6	17.52	1	1	1	1
4	8.1	6.0	5.4	6.50	8.3	22.4	21.7	17.46	4	5	4	4
5	11.4	7.2	12.3	10.30	9.7	15.3	24.1	16.36	1	1	0	0.6
6	10.2	9.1	8.9	9.40	6.3	20.5	14.4	13.73	2	1	1	1
7	13.7	7.2	9.9	10.26	16.0	25.1	34.8	25.3	1	1	1	1
8	15.8	10.7	8.6	11.70	3.0	10.1	11.0	8.03	2	1	2	1
9	7.6	8.3	4.2	6.70	11.3	10.8	9.2	10.43	4	3	8	5
10	14.1	9.0	4.8	9.30	9.9	6.0	27.8	14.56	0	1	5	2
11	12.0	7.4	7.7	9.03	1.1	14.5	35.6	17.36	2	3	3	2
12	7.5	4.5	9.5	7.16	2.8	21.4	21.0	15.06	3	3	3	3
13	9.8	7.1	5.9	7.60	12.9	37.0	31.4	27.10	2	4	4	3
14	4.5	6.7	7.6	6.26	5.9	21.2	41.7	22.93	4	5	3	4
15	3.9	3.3	3.7	3.30	9.7	35.7	9.4	18.26	6	8	9	7
16	8.2	7.3	7.0	7.50	6.0	25.8	24.6	18.80	0	3	0	1

On these criteria, Treatments Nos. 2 and 15 are significantly better than the control at the 5 per cent. level and Treatment No. 1 is significantly better at the 1 per cent. level.

The pasture on which the experiments were situated was infested with both *Oxycanus cervinatus* and *Odontria zealandica.* The damage done to the pasture by the root-feeding larvae of *O. zealandica* could affect the percentage cover gradings by which was assessed the response of *O. cervinatus* to the treatments. The data on larval populations of *O. zealandica* are therefore included in Tables I, II and III for reference. The treatments were not expected to affect *O. zealandica,* and there is no indication that they did so.

The vegetation cover gradings correlate reasonably well with the surviving *Oxycanus* population and there are no obvious anomalies in this correlation on individual plots which had large populations of *O. zealandica.*

Another factor which may account for anomalous results is the possible migration of *Oxycanus* larvae due to starvation, from control plots, untreated areas or plots on which the treatments were ineffective, on to plots where the treatment was effective and resulted in a greater vegetation cover.

Whatever the difficulties in interpretation of the *Oxycanus* population figures, the final criterion of effectiveness of the treatments must be the persistence or recovery of the pasture. Judged on this basis, it is evident that the D.D.T. spray gave results as good as the Paris Green-bran bait which was used as a standard. Gristed oats was noticeably better than the controls but not as good as Paris Green-bran or D.D.T. The other indication which is of some interest is that the addition of wheat germ to both sawdust and chaff appears to improve the attractiveness of these normally unattractive materials.

TABLE 2. EXPERIMENT II. D.D.T. DUSTS

Treatment No.	No. of living *Oxycanus* caterpillars per sq. ft.			Treatment Mean	No. of living *O. zealandica* larvae per sq. ft.			Treatment Mean	Grading of Vegetation cover after treatment.			Treatment Mean
	Block. A	B	C		Block. A	B	C		Block. A	B	C	
1	0.1	0.7	1.0	0.60	24.6	23.7	1.8	16.7	10	10	10	10
2	7.1	10.7	9.4	9.06	23.2	8.5	1.6	11.1	0	2	1	1
3	0.0	0.0	0.0	0.0	31.5	6.8	1.5	13.26	10	11	10	10
4	0.6	0.0	1.1	0.56	11.7	23.1	2.5	12.43	12	10	10	10
5	1.5	1.0	2.2	1.56	15.6	12.6	5.0	11.06	9	10	9	9
6	4.9	3.5	5.3	4.56	20.0	8.1	4.1	10.73	7	8	6	7

An analysis of variance on this section in Table II dealing with *Oxycanus* population shows the existence of significant treatment effects. The differences in treatment means required for significance are 1.65 on the 5 per cent. level and 2.35 on the 1 per cent. level.

All treatments are significantly better than the control on the 1 per cent. level. Only Treatment No. 6 (15 lb. D.D.T. per acre) gave significantly poorer results than the standard Paris Green-bran bait. Differences between the three other D.D.T. dosages were not significant.

The treatment means for *Oxycanus* population correlate well with those for vegetation cover.

Vegetation cover gradings in excess of 10 in Table II indicate that the plant cover on these plots was better than that on the Paris Green-bran plots which were taken as the standard for comparison.

TABLE III. EXPERIMENT 3. GAMMEXANE DUSTS

Treatment No.	No of living *Oxycanus* caterpillars per sq. ft.			Treatment Mean	No. of living *O. zealandica* larvae per sq ft.			Treatment Mean	Grading of Vegetation cover after treatment			Treatment Mean
	Block. A	B	C		Block. A	B	C		Block. A	B	C	
1	7.4	5.1	4.1	5.52	8.5	7.9	1.4	5.93	7	7	5	6
2	11.0	9.8	8.8	9.86	7.2	4.3	2.9	4.80	1	1	1	1
3	1.7	1.1	1.9	1.56	12.0	2.6	8.7	7.76	10	10	9	9
4	3.8	4.4	3.1	3.76	9.2	16.1	1.6	8.96	9	7	9	8
5	10.1	6.7	6.6	7.80	11.9	2.2	6.0	6.70	4	5	4	4
6	11.7	7.9	6.8	8.80	8.8	12.0	3.2	8.00	3	1	1	1

An analysis of variance on the section of Table III dealing with the *Oxycanus* population discloses that there are significant treatment effects. The differences between means required for significance are 1.98 on the 5 per cent. level and 2.82 on the 1 per cent. level.

Treatments Nos. 3 and 4 are significantly better than the control on the 1 per cent. level, Treatment No. 5 is significantly better on the 5 per cent. level and Treatment No. 6 is not significantly better than the control. The *Oxycanus* populations from Treatment No. 1 (the standard Paris Green-bran bait) are much higher than is usual with this treatment, but no cause can be assigned for this unless it is due to migration of larvae from other plots.

DISCUSSION

Experiment 1.

The treatments for trial were compared with an untreated control plot and with a standard Paris Green-bran bait.

The wettable D.D.T. (Treatment No. 2) used at a dosage of ½ lb. of the para para isomer in 100 gallons of water per acre gave excellent results. The cost for material alone of this treatment would be approximately 9/- per acre.

The Paris Green and gristed oats treatment (No. 4) which gave a partial control, would cost for materials alone approximately 14/- per acre. This is based on Paris Green at 3/- per lb., oats ex store at 5/6 per bushel, plus cost of gristing. This bait besides being less effective and more costly than the Paris Green-bran bait, is difficult to mix properly owing to the balling of the fine floury fraction and probably would not run as freely as bran through a top-dresser. Using only the coarser fraction of the gristed oats (Treatment No. 15) which was retained on a $\frac{1}{16}$ inch mesh sieve and constituted approximately half the weight of the whole sample, the cost would be increased to approximately 20/- per acre without any noticeable increase in efficiency.

Treatments Nos. 5-9 and 10-14 constituted an attempt to increase the attractiveness of sawdust and chaff, both of which are unattractive. Since it was considered possible that vitamin B_1 or associated vitamins of the B group might be in part responsible for the attractiveness of bran, four materials of different B_1 content were added to these carriers. The vitamin B_1 content of these was as follows :—

White flour (72 per cent. extraction), 1.7 gammas ; 80 per cent. flour, 3.0 gammas ; wheat germ, 15.0 gammas ; high B_1 flour, 20-25 gammas. Of these materials, wheat germ alone showed any promise and as this was not the highest in B_1 concentration it is presumably some other constituent which is the attractive principle. It has not so far been possible to test the influence of the fats and oils normally occurring in wheat germ and bran on the attractiveness of baits. Wheatgerm is quoted at the same price as flour—about 1.65 d. per pound.

Experiment 2.

In this experiment D.D.T. applied as a dust was tested at different dosages against the standard Paris Green-bran bait.

The results demonstrated that satisfactory control of *Oxycanus* could be secured with quantities as low as 30 lb. per acre of 2 per cent. D.D.T. dust. The results from the 15 lb. per acre dosage were noticeably poorer. This dust was a jet-pulverised dust containing 2 per cent. of the para para isomer of D.D.T and diluted with equal parts of talc-magnesite and china clay. The talc-magnesite is 60 per cent. talc and 40 per cent. magnesite. This is a dust of very fine particle size. Estimating the visible coverage by the dust after application, it was considered that 120 lb. per acre was wastefully heavy, 60 lb. was a good coverage, 30 lb. was getting too light, and 15 lb. was too light.

The results are more instructive if expressed in terms of para para isomer content. For Treatments Nos. 3, 4, 5 and 6 these are 2.4, 1.2, 0.6 and 0.3 lb. per acre. The lowest satisfactory dosage of 30 lb. per acre or 0.6 lb. per acre of the para para isomer gave similar results to the D.D.T. spray at 0.5 lb. para para isomer per acre in Experiment 1.

This 2 per cent. dust is retailed at 9d. per lb. and at a dosage of 30 lb. per acre would cost 22/6 per acre for materials alone. The necessity for specialised dusting equipment for large scale application is an additional disadvantage.

Experiment 3.

In this experiment the treatments were not applied until 2 weeks after those in Experiments 1 and 2. As the treatment had 6 weeks in which to act it is fair to assume that it had exerted its full effect and that it can be legitimately compared with results from other treatments in Experiments 1 and 2. The gammexane dust gave effective control of *Oxycanus*, but at a relatively higher dosage per acre than the D.D.T. dusts. This dust was given as a 4 per cent. dust containing 0.5 per cent. gamma isomer. The price was quoted as approximately ¼d. per lb. If Treatments Nos. 3, 4, 5 and 6 are expressed as pounds of gamma isomer per acre, these are 0.6 lb., 0.3 lb., 0.15 lb. and 0.075 lb. A dosage of 120 lb. per acre (0.6 lb. g.i.) produced results equivalent to those from 30 lb. per acre (0.6 lb. p.p.i.) of D.D.T., D.D.T. and gammexane in equal dosages of the active isomers appear to be equally toxic to *Oxycanus*. This formulation of gammexane, at a lower active isomer content and a higher cost per pound, is not as economical as D.D.T. It suffers from the same disadvantage as D.D.T. dust in requiring special equipment for application.

CONCLUSIONS

No satisfactory alternative to bran, for use as a carrier in poison baits has been discovered. The use of gristed oats showed some promise, but this material is dearer than bran and subject to disadvantages in mixing and spreading which are not encountered with bran.

Wheat germ showed some promise as an adulterant for otherwise unattractive carriers such as sawdust and chaff, and efforts should be made to isolate and identify the attractive principle in bran and wheat germ, if indeed attractiveness is conferred by a single substance.

D.D.T. and gammexane dusts appeared to be equally effective weight for weight of their active isomers. D.D.T. has an advantage in cost over gammexane, but both require special dusting equipment. A D.D.T. spray at 0.5 lb. of the para para isomer in 100 gallons of water gave effective control at a cost, for materials alone, little in excess of the standard Paris Green-bran bait. If efficient spraying and dusting appliances were available, sprays would probably be preferred on the score of cost but this may be offset by the volume of water required for spraying large areas of pasture. The application of D.D.T. concentrates as aerosols, using very much smaller volumes of liquid per acre, would appear to have considerable promise.

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STORAGE OF CURD FOR PIG-FEEDING

BIOCHEMICAL INVESTIGATIONS

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Summary

To study the biochemical factors involved, curd has been prepared from buttermilk and stored in twenty-one containers of varying sizes for periods of six to eight months. By detailed chemical analyses of the buttermilk, of the curd and especially of the whey produced from it, the progress of changes during preparation and storage has been followed. The results have made clear the basic principles on which practical methods of preparation and storage depend for success. The main bacteriological and chemical changes appear to be quite similar to those which occur in traditional methods of cheese-making.

INTRODUCTION

DURING recent years an increased interest has been taken, especially in northern dairying districts, in the storage of curd made from buttermilk and skim-milk for the purpose of having supplies of this valuable food available in winter for pig-feeding. To provide winter food for both man and animal, various methods of storing dairy produce have been used in many countries from time immemorial. According to Arthur Young, F.R.S., a pioneer of scientific farming, the storage of skim-milk and whey in brick cisterns appears to have been practised in the eighteenth century. In his eighth edition of "The Farmer's Calendar", published in 1809, he recommended this method of storing dairy by-products to provide winter feed for pigs farrowed in the autumn.

Notwithstanding the antiquity of the process, the idea arose in recent years that some very special methods were required to initiate successfully the preparation and storage of curd. This idea was fostered by the fact that some operators stored curd year after year without a failure, while others sometimes succeeded, but at other times failed. Consequently scientific assistance was solicited to study the biochemical problems associated with the preparation and storage of curd from buttermilk or skim-milk. During the 1944-45 dairy season, some preliminary investigations were undertaken at Featherston, and the results of these enabled plans to be made for an extensive series of trials during the 1945-46 season. These trials were carried out at the farm where garbage from the Japanese prisoner-of-war camp was being used for feeding pigs, and where also an ample supply of buttermilk from the local dairy factory was available for use.

The formation or precipitation of curd from buttermilk or skim-milk obviously results from bacterial fermentation of milk sugar, yielding chiefly lactic acid. This dissolves the lime salts naturally combined with casein, which is then precipitated and gradually aggregates together into curd. At first sight it might have appeared desirable to study the bacteria responsible for these changes to try to find out if any particular types were more suitable than others for the purposes desired. However from a knowledge of the biochemistry of milk, it was clear that a variety of milk-souring bacteria were capable of producing such changes, and the results finally yielded by using different types of bacteria might not show quite as much variation as would be expected. The variations which might occur were chiefly in the time taken to complete the souring process, and those times were also related to the optimum growth temperatures of specific organisms. A knowledge of past researches in dairy bacteriology also indicated that a tremendous amount of work would be required to isolate, and to study one by one in pure culture, various types of milk-scouring bacteria, to find which might be the best for the purpose of preparing curd for storage. Apart from these considerations, the original buttermilk would obviously have a liberal inoculation of a considerable variety of bacteria. It was therefore decided that to attempt any detailed bacteriological work on the curd during preparation and storage would be unprofitable, but instead plans were made to use suitable methods of chemical analysis to follow the changes produced by bacteria. The only bacteriological part of the investigation consisted in providing a substantial inoculation of certain lots of buttermilk with specific types of lactic acid bacteria to find the effect upon the process and the finished product. To enable comparisons to be made a series of containers of various sizes were procured in which to prepare and store curd under various conditions. The lay-out of these containers is shown in the first illustration accompanying the practical description already published (1). A programme of analytical work was mapped out, and although the preparation of curd was the ultimate object of the trials, much useful information was obtained by detailed analysis of other materials, particularly the whey associated with curd. During the course of the trials, samples as required were taken to the Dairy Laboratory, Wallaceville, to be analysed. The analytical methods used are described in an appendix at the end of this paper.

OUTLINE OF TRIALS

The containers used for the *first* series were ten 50-gallon iron drums in five pairs labelled A and B, C and D, E and F, G and H, L and M, and one 80-gallon wooden cask, labelled W. Four of these, B, D, F and H were sunk to nearly their full depth into the ground and the remainder were left standing on the surface. These drums were unprotected except that for the first three months they were on the sheltered side of small army huts which were later removed. The sunken drums were covered over in the later stages to prevent farm animals getting at them. The drums were first filled and inoculated on the 10th October, 1945, and further additions of buttermilk were made on the dates given in Table I, which also summarizes the trial by giving the dates on which curd and whey samples were obtained, in each case before the removal of whey to make room for the addition of further buttermilk.

The first pair A and B, were used as uninoculated controls and to all the others varying proportions of ordinary cheese starter (*Streptococcus cremoris*) were added, while G and H, L and M, and W were also inoculated with *Lactobacillus bulgaricus*. Inoculations of 1-2 gal. were used for each drum, to ensure rapid souring. The controls soured and fermented in the same manner as the inoculated drums, but previous experience of other investigators showed that this might not always occur. The buttermilk used was obtained from the Featherston Dairy Factory, close to the farm on which the trials were carried out. As it was transported in a mobile tank also used for handling whey from the cheese vats, it would pick up a certain amount of lactic cheese starter, and that probably explained the successful souring of the uninoculated controls. The progress of the initial souring was observed by titrating so as to give results expressed conventionally as " per cent. lactic acid ", The results at the end of the first forty-eight hours showed that souring in the sunken drums was taking place at a noticeably slower rate, for the figures were 0.45 to 0.51 per cent. as compared with figures of 0.60 to 0.73 per cent. for the rest. Lower underground temperatures would account for this. After three days the slower drums had caught up with the rest and all the figures were in the range 0.72 to 0.85.

Besides lactic acid, carbon dioxide was formed in considerable quantities, and as this gas rose to the surface it gradually took with it the curd, so that after a few days, there was on top of each drum a layer of curd several inches thick, buoyed up by the accumulated gas. Underneath was the whey which gradually drained out of the curd so that the latter became drier and firmer. In order to get the curd covered with whey it was broken up from time to time, until the curd gathered at the bottom, leaving the upper portion of each drum filled mainly with whey. In the early stages, the surface curd layer formed again after each addition of buttermilk, but later, when further buttermilk was added, the acidity of the mass was sufficient to precipitate the new curd immediately, so that it gathered upon that already accumulated at the bottom of each container before gas formation could occur. In the latter stages only odd lumps floated up and these sank on being broken to allow the gas to escape. Finally the drums were about four-fifths full of curd, covered by a few inches of whey. The product was a rather sloppy greyish-white mass which, when drained of surplus whey gave a soft granular curd.

TABLE I. FIRST SERIES—DATES OF OPERATIONS, 1945-46

Buttermilk.		Curd.		Whey.	
Added	Analysed.	Broken.	Analysed.	Removed.	Analysed.
10/10	10/10				17/10
		27/10			27/10
5/11*	5/11	5/11	5/11	5/11	5/11
12/11			12/11	12/11	12/11
15/11	15/11	14/11		15/11	
19/11	19/11	18/11			
			28/11		28/11
6/12	6/12			5/12	5/12
					12/12
18/12					18/12
(W only)			29/12		29/12
			4/2		4/2
			4/3		4/3
					21/3
E and F omitted			8/5		8/5

The whey samples of 17th and 27th October were obtained from under the curd by piercing it and thrusting a closed bottle down into the whey where the bottle was allowed to fill. After the curd had been broken on the 5th November the whey samples were taken by immersing a bottle in the surface layer. If much suspended curd entered the bottle the sample was allowed to stand so that the curd sank and portions for analysis were removed from the supernatant layer. From 4th March onwards the whey samples were obtained by filtering portions of wet curd through a coarse filter paper for about three hours. In sampling curd from under the whey all surplus whey possible was drained from the sample while it was being bottled. Until the analyses were completed all samples were kept in a cold room at a temperature of 38° to 40°F.

The *second* series was begun on 28th November, 1945, using eight wooden casks each holding about 46 gallons, in four pairs, labelled O and P, Q and R, S and T, Y and Z, also a larger cask X, holding about 80 gallons, and a large wooden vat V of total capacity about 470 gallons. All of these were above the ground and right out in the open except V which was for about six weeks shaded by a small shed. Prior to filling with buttermilk they were all well scrubbed out and rinsed with a solution of chloride of lime. With the exception of O and P, which were controls, they were inoculated with mixed cheese starters *S. lactis* and *S. cremoris*, which were added to all the rest except X, to which *L. acidophilus* alone was added. To S and T and V some *L. bulgaricus* was added while to Y and Z some *L. acidophilus*. Inoculations of about 1 gal. were used except in V where a total of about 8 gal. was used. The vat V was at first only half-filled as it was inclined to leak, but later was about three-quarters filled. Some leakage continued from this vat and also from two or three of the casks.

The story of the remainder of the process is very much the same as in the first series. Warm sunny weather stimulated gas production so that the curd rose more quickly than in the first series, and the gas threatened to force the curd out of the top of the casks so it had to be broken up earlier than in the first series. Whey was removed and more buttermilk added, as indicated in Table II, the intervals being more uniformly spaced than in the first series. Before the curd was first broken up it had become slightly compacted in the narrow top of each cask so that its moisture content was lower. Later samples of curd from under the whey gave moisture figures similar to those from the first series. The curd itself had a rather whiter appearance than that of the first series, due to the absence of iron.

TABLE II SECOND SERIES—DATES OF OPERATIONS, 1945-46

Buttermilk.		Curd.		Whey.	
Added	Analysed	Broken.	Analysed.	Removed.	Analysed
28/11	28/11				
6/12	6/12	5/12	5/12	5/12	5/12
12/12*	12/12	10/12		12/12	12/12
18/12	18/12	19/12† 20/12†		18/12	18/12
31/12*	31/12			29/12	29/12
10/1			9/1	9/1	9/1
			4/2		4/2
			4/3		4/3
					21/3
10/4 (V only)					17/4 (V only)
			8/5		8/5

* Except P, R, T and Z

† Where necessary

In discussing the results, reference is made to the effects of varying weather conditions. The drums and casks were left unprotected from weather changes with the object of carrying out the trials under conditions which would resemble as closely as possible the general conditions which would apply on most farms. Thus the information derived would be of more value to those intending to try to preserve curd by the methods studied.

TABLE III. BUTTERMILK ANALYSES

Date of Sample.	10/10/45*	5/11/45†	15/11/45§	19/11/45	28/11/45†	28/11/45§	6/12/45§	12/12/45	18/12/45	31/12/45
Spec. Grav. at 60°F.	1.0273	—	—	1.0252	1.0279	—	—	1.0268	1.0241	1.0248
Total Solids Per Cent.	7.52	8.25	6.13	7.13	7.62	7.53	7.62	7.23	6.73	6.92
Total Nitrogen Per Cent.	0.412	0.431	0.342	0.395	0.424	0.412	—	0.353	0.310	—
Total Protein Per Cent. (T.N. × 6.38)	2.63	2.75	2.18	2.52	2.71	2.63	—	2.25	1.98	—
$\frac{N}{T.S.} \times 100$	5.48	5.22	5.58	5.54	5.56	5.47	—	4.88	4.61	—
Lactose Per Cent.	3.67	3.86	1.18	1.87	2.98	3.20	2.76	3.22	3.07	3.09
Chloride as Cl^-ion Per Cent.	0.0694	0.0653	0.0651	0.0669	0.0693	0.0735	0.0746	0.0714	0.0694	0.0634

* Average of results for three samples. † Average of results for two samples. § Sample sour.

RESULTS OF BUTTERMILK ANALYSES

As the buttermilk was the starting material a fairly complete analysis of most of the samples was done to provide a basis for considering subsequent changes. The analyses were begun as soon as possible after sampling to avoid changes, but in some cases the sample became more or less sour so that certain values were affected, e.g., the total solids and lactose were both very low in the sample of 15/11/45. The results summarized in Table III indicate the composition of the buttermilk used over a period of three months.

(a) Specific Gravity variations were due to the fact that water was added to the cream for rinsing and other purposes during butter manufacture, so that the resulting buttermilk was diluted to a varying extent. Most of the samples had a specific gravity of about 1.027, but one sample with more water than usual had a specific gravity of 1.024 and total solids of 6.73 per cent.

(b) Total Solids figures were usually in the vicinity of 7.5 per cent., the variation being more or less parallel with the specific gravity and nitrogen content. Samples with lower lactometer readings due to added water gave lower values ; due to loss of lactose, sour samples were also lower in total solids. For lack of time, fat determinations were not done.

(c) Lactose figures ranged from 3.2 to 3.8 per cent. for samples analysed before souring except one apparently fresh sample which had the unusual value of 1.9 per cent. (with T.S. 7.1 per cent.).

(d) Total Nitrogen was determined to give an indication of the feeding value of the buttermilk, and since the resulting curd consists mainly of coagulated protein the figures can be used to indicate the degree of concentration of the protein during the process. The total nitrogen ranged from 0.35 to 0.42 per cent. with one or two exceptions. Using the accepted factor of 6.38 to convert these figures to protein, a range of 2.2 to 2.7 per cent. protein was obtained. On the earliest samples, protein partition determinations were done to get an indication of the distribution of the protein. On samples with a total nitrogen of 0.41 per cent. the results showed that of this, 0.385 per cent. was total protein (including nitrogen, 0.35 per cent. as casein nitrogen) and leaving only 0.025 per cent. as non-protein nitrogen. This gave a casein percentage of 2.2 and a soluble protein percentage of about 0.22, indicating a casein to soluble protein ratio of 10 to 1 as compared with about 8 to 1 in whole milk. The difference was due to the denaturation of the protein during flash-pasteurizing of the cream at 190° to 200°F.

As a check on apparent variations, a nitrogen to total solids ratio was derived. For most of the samples a value of about 5.5 was obtained indicating a fairly uniform composition for the buttermilk. Slightly lower ratios were obtained for later samples, but no reason for this could be suggested.

(e) Chloride determinations were done as a control to indicate subsequent changes. The results vary within the narrow range of 0.065 to 0.072 per cent. A weighted average of all the results gave a value of 0.0687 per cent. of chloride ion. By converting all subsequent whey results to this chloride basis the effects of both evaporation and rainwater dilution were eliminated, as is discussed later.

Most of these results are only indirectly related to the curd and whey results, except that they indicate the original composition of the material used to prepare the curd. The lactose is important on account of the part it plays as the source of lactic acid, etc. From the results can be obtained an estimation of the original feeding value of the buttermilk, and if desired theoretical figures for the yield of curd from a given amount of buttermilk. The composition of the buttermilk depends on the methods used at the dairy factory, and varies appreciably from day to day, but excessive dilution with water should be avoided when it is to be used for pig-feeding.

RESULTS OF WHEY ANALYSES

Associated with the curd was a considerable volume of whey which at first sight might have appeared to be a by-product of large bulk but of little significance. It was however important in several respects, and from its analysis was derived much very useful information about what was happening to the curd. The progress of souring and subsequent changes could be followed by acidity and pH determinations. During storage the lactose was gradually fermented and by lactose determinations the progress of fermentation, whether faster or slower, could be followed. The whey contained nitrogen in various forms about which analytical data might prove useful. Besides nitrogen compounds of lower molecular weight, there might also be soluble milk proteins or their derivatives which could not be precipitated like casein to form curd. Finely dispersed curd which had not become aggregated with the main bulk might also be determined as nitrogen when the whey was analysed soon after souring, or after the addition of more buttermilk. Later on if the curd decomposed during storage the nitrogen content of the whey should show an increase.

The total solids present in the whey included such variables as lactose and nitrogen compounds so that determinations of total solids could provide a general indication of the trend of changes which were occurring during storage. A minor constituent which could be quickly and easily determined was chloride and estimations of this ion proved very useful for control purposes.

The whey for the earlier analyses was obtained from the surplus which was being removed to enable more buttermilk to be added and was usually very gassy. Later on the curd almost filled the drums and casks, with only a small layer of whey on top and little or no gas. At that stage the whey samples were drained from curd taken deep down in the drums so as to be less subject to surface variations. As all the casks were out in the open, the composition of the whey was affected by climatic effects, which operated in three ways. Firstly, the drums let into the ground were kept cooler than those above ground, and secondly, especially in hot windy weather, the whey evaporated more readily from the surface. As the sunken drums were also somewhat sheltered from the wind, these two conditions together gave rise to the markedly lower chloride values yielded by samples from the sunken drums B, D, F and H of February 4th.

This is shown in the following figures which are per cent. of Cl ion :—

A, 0.103	C, 0.121	E, 0.114	G, 0.136	L, 0.135	W, 0.103
B, 0.094	D, 0.075	F, 0.081	H, 0.098	M, 0.124	

Thirdly, dilution occurred in wet weather. The extent of the variations produced by climatic effects was shown by two sets of samples taken at the same time from both series. In the first set which was chiefly surface whey, the chloride figures ranged from as low as 0.023 to 0.099 with an average of 0.0551 per cent. From deep curd samples taken at the same time the whey was drained and yielded chloride figures from 0.075 to 0.099, with an average of 0.0862 per cent.

All whey results recorded in Tables IV and V were converted to a value corresponding to the mean chloride concentration in the buttermilk used—0.0687 per cent. The chloride content of the initial whey was much the same (0.066 per cent.), but the average chloride figure from the buttermilk samples was preferred as the basic figure. By this means variations due to evaporation and dilution effects were largely eliminated, as is best shown in the graphs, Fig. 2. The uncorrected values for total solids of L on 12th December and 4th February were higher than they should have been, probably owing to evaporation by hot winds. In the case of S, slight dilution due to wet weather is shown in the values for 29th December. On the same date the values for V showed much more marked dilution due to rain water being accidentally blown into V from the roof of an army hut a few days before. On 10th April additional buttermilk was added to V to keep the curd covered and to replace loss due to leakage etc., a whey sample taken on 17th April gave a total solids value showing that the additional buttermilk had somewhat modified the whey composition. To supplement the graphs, in Tables IV and V are given the principal analytical results for whey for most of the containers. For brevity the remainder, which were similar, have been omitted.

As already mentioned acidity titrations were done to follow the initial souring of the buttermilk, but the results obtained from titrating the whey were rather variable, and latterly became very high, so that little significance could be attached to the figures. The pH of the whey was more constant. Soon after the buttermilk soured the values of the first series were 4.4, but a week or two later they dropped to 4.2, where they remained fairly consistently for most of the time. In the second series similar values were recorded, but after some months' storage, individual samples gave more variation, from 4.0 to 4.6.

First Series : (a) Lactose was used up by the initial bacterial fermentation to produce lactic acid, carbon-dioxide, etc. Determinations of lactose therefore gave an indication of the progress of fermentation, and after all the lactose had gone, showed when other changes might begin. The results have been expressed graphically (Fig. 1) by plotting the lactose concentration against the time in days, using lactose value for the first buttermilk as a starting point. As each further addition of buttermilk temporarily increased the lactose content, the lactose figures for the buttermilks have also been plotted as starting points, thus breaking the graphs into a series of smaller graphs joined by dotted lines. This eliminated those portions of the main graph which showed that lactose was being added, and gave a better indication of the rate of fermentation of the lactose. Points are shown for buttermilk additions on 12th and 15th November but as reliable lactose values for these were not available, lines from these points have not been drawn.

In Fig. 1 are graphs for A and B, and E and F ; similar graphs with minor variations were obtained for the other drums. In all cases the lactose was destroyed fairly rapidly, but the drop appeared irregular

TABLE IV. WHEY ANALYSES. FIRST SERIES

The figures represent Total Solids, Lactose and Total Nitrogen percentages.

Sample.	Sample dates.	17/10	27/10	5/11	12/11	28/11	5/12	12/12	18/12	29/12	4/2	4/3	21/3	8/5
A	Total Solids	4.87	4.49	4.36	4.42	3.52	3.06	3.31	2.93	2.64	2.90	2.71	2.76	2.75
	Lactose	2.45	1.25	0.94	1.60	0.23	0.07	0.35	0.09	0.00				
	Nitrogen	0.108					0.082	0.075		0.068	0.104	0.118		0.127
	*Formol							1.27	1.44		2.06	2.36	2.39	3.01
	*Ratio, Formol to Nitrogen							16.9			19.8	20.0		23.7
B	Total Solids	4.23	4.58	4.51	4.68	4.18	3.80	4.26	3.74	3.06	2.93	2.72	3.11	3.10
	Lactose	2.46	1.81	1.23	1.59	1.26	1.05	1.49	1.13	0.00				
	Nitrogen	0.075					0.068	0.073		0.085	0.084	0.113		0.137
	*Formol							1.01	1.18		1.47	1.95	2.40	2.69
	*Ratio, Formol to Nitrogen							13.8			17.5	17.3		19.6
E	Total Solids	4.71	4.57	4.42	3.98	2.94	2.61	2.86	2.70	2.21	2.36	2.34	1.81	1.75
	Lactose	2.74	2.04	1.45	0.15	0.00	0.00	0.17	0.00	0.00				
	Nitrogen	0.104					0.069	0.075		0.080	0.092	0.120		0.133
	*Formol							1.36	1.49		1.87	2.96	2.96	4.36
	*Ratio, Formol to Nitrogen							18.1			20.3	24.7		32.8
F	Total Solids	4.79	4.55	4.50	4.38	4.03	3.68	4.03	3.74	3.08	2.95	2.46	3.19	2.74
	Lactose	2.72	1.78	1.20	0.51	1.19	0.82	1.47	1.07	0.00				
	Nitrogen	0.107					0.071	0.069		0.083	0.089	0.092		0.147
	*Formol							0.97	1.12		1.69	1.83	2.30	3.69
	*Ratio, Formol to Nitrogen							14.1			19.0	19.9		25.1

TABLE IV. (Continued.)

L	Total Solids	4.64	4.73	4.33	4.69	3.01	2.59	3.73	3.41	2.74	2.62	2.61	2.89	2.80
	Lactose	2.79	2.03	1.82	2.00	0.37	0.00	1.53	1.06	0.00				
	Nitrogen	0.102					0.077	0.070		0.093	0.102	0.139		0.135
	*Formol							1.28	1.49		1.94	2.80	2.74	3.26
	*Ratio, Formol to Nitrogen							18.3			19.0	20.1		24.1
M	Total Solids	4.86	4.68	4.57	4.65	3.39	2.73	3.41	3.07	2.49	2.62	3.12	3.00	2.77
	Lactose	2.84	1.94	1.75	1.75	0.21	0.00	0.86	0.36	0.00				
	Nitrogen	0.102					0.074	0.075		0.079	0.090	0.120		0.119
	*Formol							1.14	1.41		1.68	2.50	2.34	2.73
	*Ratio, Formol to Nitrogen							15.2			18.7	20.8		22.9
W	Total Solids	4.69	4.80	5.16	4.58	3.54	2.98	3.99	3.75	2.99	2.83	2.90	3.44	3.20
	Lactose	2.83	2.13	2.32	1.63	0.21	0.18	1.84	1.44	0.12				
	Nitrogen	0.102					0.089	0.076		0.056	0.105	0.163		0.189
	*Formol							1.44	1.57		2.18	3.02	3.38	4.40
	*Ratio, Formol to Nitrogen							18.9			20.8	18.5		23.3

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* Analytical methods for determining Formol values and Formol to Nitrogen ratio values are described in the Appendix.

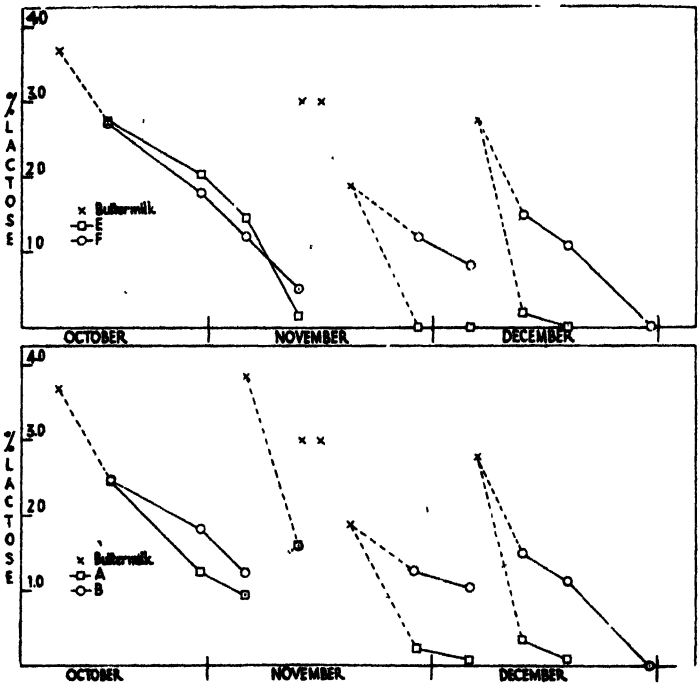

FIG. 1.- -To show changes in lactose content of whey.

owing to the increase due to more buttermilk being added. Once addition of buttermilk ceased the lactose soon became completely used up.

In these graphs, a significant fact is that for A and E (above ground) the lactose fell off rapidly and further buttermilk did not maintain the lactose content for very long. In B and F the drop was slower and lactose was present in larger amounts for much longer after each addition of buttermilk. This was no doubt due to the lower temperature in the underground drums slowing down the rate of fermentation – an important point.

(b) Total solids at first ranged mostly from 4.5 to 4.9 with an average of 4.7 per cent. then gradually dropped as the lactose was used up, with slight rises after more buttermilk was added, (e.g., Fig. 2, graph L, points for 12th November and 12th December) until, with the loss of all the lactose, low values were reached. Except for slight irregular variations, values then remained fairly constant in the vicinity of 2.5 to 3.0 per cent. This suggests that very little curd was dissolving in the whey. In W, however, after the initial low value was reached there was a slight but distinct rise in total solids from 2.8 to 3.2 per cent. Similar observations were made in the second series and the explanation will be discussed later.

(c) Total nitrogen determinations could not be done on several early batches of samples and the picture is therefore incomplete. The initial values given by most of the first samples were close to 0.10 per cent., and these rather higher values were probably due to the presence of finely dispersed casein which had not become aggregated with the bulk of the curd. Later figures in December, before and after the last addition of buttermilk, were lower, (about 0.07 to 0.08 per cent.). The values then increased only slightly to about 0.09 per cent. in February, then to about 0.12 per cent. in March and finally to about 0.14 per cent. on 8th May. In W a greater increase was noticed, as with the solids figure, and that again was similar to the second series.

To indicate the progress of curd breakdown and solution in the whey, "formol" titrations of the available amino-groups were done on all samples in the later stages. The results of these are shown in Table IV where figures represent ml. of 0.1 N. sodium hydroxide per 10 ml. of whey, and are adjusted to a fixed chloride content, Cl = 0.0687 per cent. The values obtained for samples taken after the addition of buttermilk had ceased, over a period of more than five months, rose from a range of about 1.0 to 1.4 ml. to a range of 2.9 to 4.4 ml. This distinct rise showed that breakdown to the curd to give soluble nitrogen compounds was taking place though quite slowly. In the case of W there was a more marked rise, parallel with the nitrogen and other values mentioned above. Most of the formol titrations for the sunken drums did not rise to quite the same extent as the remainder so that cooler temperatures apparently retarded decomposition slightly. To illustrate the increasing proportion of amino-nitrogen in the whey, the ratio, formol titration to total nitrogen, has been calculated and, with odd exceptions, most of the figures so obtained showed a gradual increase over the time considered.

(d) Chlorides were done as a control and their value in eliminating the effects of the weather has been shown. At first they remained fairly constant at about 0.066 per cent., but gradually rose to 0.08 to 0.12 per cent. (average 0.094).

Second Series: As the containers used were wooden casks above the ground together with a wooden vat, they did not get so hot in the sun as the iron drums, but some of them leaked a little.

(a) Chloride figures showed a rise similar to that observed in the first series, from 0.067 per cent. originally to 0.07 to 0.09 per cent. (average 0.081 per cent.) at the last. All the results in Table V, have been converted to a chloride concentration of 0.0687 per cent. and only the adjusted figures are discussed.

(b) Lactose results showed a gradual fall similar to that in those of the first series standing above the ground, but the additions of more buttermilk at frequent intervals checked the fall in values. In most cases the lactose was all fermented at the end of two months, twenty days after the addition of the last buttermilk.

(c) Total solids values were at first in the range 4.6 to 4.8 and fell gradually as the lactose was used up, while addition of buttermilk caused rises, e.g., values of 9th January for S and V shown in the graphs (Fig. 2). The V sample for that date was high in both total solids and nitrogen, due to a larger amount of buttermilk being added on 31st December. The lactose was fermenting slowly and the curd had not aggregated well owing to its gassy condition. When the lactose was practically all gone, values on 4th February ranged from 1.0 to 2.7 per cent., but during the next three months a gradual increase took place in all cases, so that the final range became 2.2 to 3.3 per cent. This is comparable with W of the first series, and is discussed later.

TABLE V. WHEY ANALYSES. SECOND SERIES

The figures represent Total Solids, Lactose and Total Nitrogen percentages.

Sample.	Sample dates.	5/12	12/12	18/12	29/12	9/1	4/2	4/3	21/3	8/5
O	Total Solids	4.68	3.72	3.37	2.88	3.11	2.58	2.90	2.93	3.16
	Lactose	2.67	1.36	0.84	0.20	0.21	0.08			
	Nitrogen	0.075	0.077		0.080	0.092	0.099	0.166		0.175
	*Formol						1.69	3 01	3.00	3.90
	*Ratio, Formol to Nitrogen						17.1	18.1		22.3
P	Total Solids	4.80	3.73	3.22	3.03	2.90	2.19	2.74	2.93	3.12
	Lactose	2.78	1.41	0.72	0.53	0.44	0.03			
	Nitrogen	0.076	0.073		0.074	0.076	0.077	0.159		0.182
	*Formol						1.56	2.88	2.90	3.58
	*Ratio, Formol to Nitrogen						20.2	18.1		19.7
S	Total Solids	4.77	3.81	3.39	2.91	3.21	2.38	2.70	2.81	3.19
	Lactose	2.74	1.41	1.04	0.55	0.66	0.09			
	Nitrogen	0.074	0.074		0.077	0.083	0.083	0.147		0.179
	*Formol						1.48	2.80	3.15	4.30
	*Ratio, Formol to Nitrogen						17.8	19.0		24.0
T	Total Solids	4.70	3.87	3.51	3.37	3.22	1.59	1.94	2.42	2.20
	Lactose	2.84	1.44	0.84	0.92	0.55	0.00			
	Nitrogen	0.074	0.074		0.071	0.075	0.077	0.142		0.153
	*Formol						1.52	2.69	3.44	4.50
	*Ratio, Formol to Nitrogen						19.7	19.0		29.4

Table V. (Continued.)

V	Total Solids	4.73	4.19	3.55	3.07	3.56	2.32	2.01	2.43	3.34
	Lactose	2.83	1.75	1.06	0.89	1.48	0.95			
	Nitrogen	0.078	0.075		0.068	0.092	0.071	0.110		0.208
	*Formol						1.44	2.23	2.74	4.60
	*Ratio, Formol to Nitrogen						20.3	20.3		22.1
Y	Total Solids	4.80	4.01	3.68	3.24	3.07	1.71	1.85	2.45	2.28
	Lactose	2.83	1.67	1.26	0.83	0.67				
	Nitrogen	0.073	0.700		0.069	0.073	0.080	0.135		0.202
	*Formol						1.04	2.70	3.42	5.45
	*Ratio, Formol to Nitrogen						13.0	20.0		27.0
Z	Total Solids	4.81	3.91	3.66	3.50	3.10	1.79	1.97	2.40	2.34
	Lactose	2.87	1.38	1.04	1.19	0.68				
	Nitrogen	0.074	0.073		0.074	0.071	0.084	0.140		0.166
	*Formol						1.74	3.08	3.10	5.20
	*Ratio, Formol to Nitrogen						20.7	22.0		31.3

* Analytical methods for determining Formol values and Formol to Nitrogen ratio values are described in the Appendix.

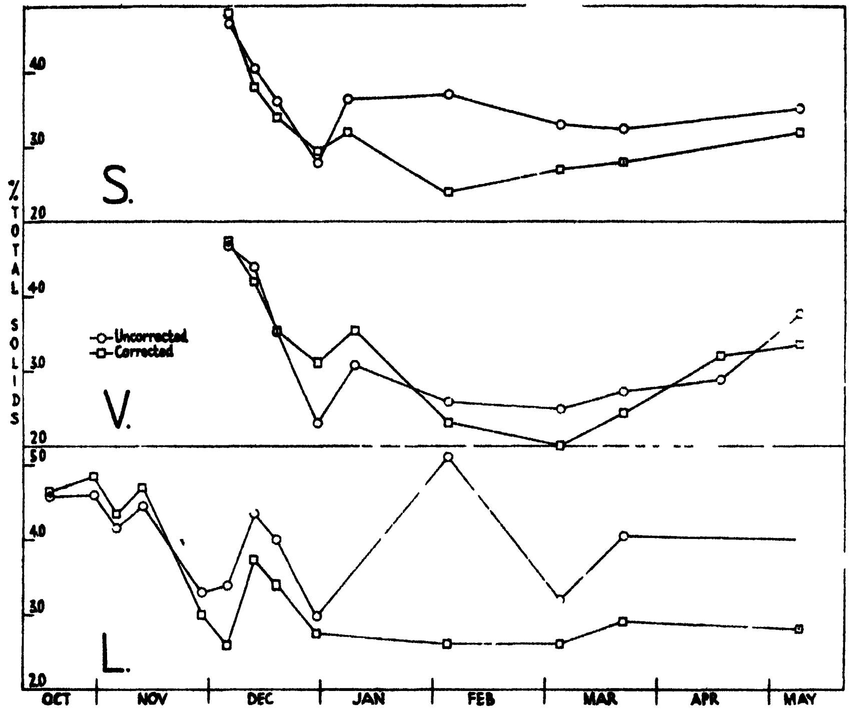

FIG 2 To show changes in total solids content of whey

(d) Total nitrogen figures are available for most of the whey samples of the second series, so that they provide a more complete picture than in the first series. While buttermilk was being added at regular intervals most of the values remained fairly constantly in the range of 0.07 to 0.08 per cent. (which agreed with the values obtained in the first series before and after the last buttermilk was added). When no more buttermilk was being added and the lactose was used up, the values rose during the last twenty weeks to figures ranging from 0.15 to 0.20 per cent. for the last batch of samples. The formol titration figures obtained after the additions of buttermilk had ceased, showed a steady rise from rather less than 2.0 ml. to a range of 3.5 to 5.5 ml. These figures and the formol titration to total nitrogen ratios obtained from them, were similar to those of the first series but the rise was more marked.

In the first series, the solids and nitrogen values of cask W latterly rose more distinctly than those of the remainder, while a similar rise was quite noticeable in all of the second series towards the end of the storage period. This difference is illustrated in Fig. 2 in which graphs of the total solids values for L, S and V, both original and adjusted, have been plotted to show the effect of the conversion factor. In L, corrected, there is a fairly steady fall (apart from rises shown in samples of 11th November and 12th December due to buttermilk additions) with eventually a gradual flattening of the graph, whilst in S and V after an initial drop there is a steady rise. The following suggestions may explain this rise which occurred in the second series but not in the first. Except for W which was a wooden cask like all of the second series, the curd of the first series was stored in iron drums, and there were indications of a relatively high iron content in the whey from these drums, e.g., the whey was a pale greenish-yellow colour, and on titrating it with alkali a blackish precipitate was obtained. Possibly the dissolved iron checked certain types of bacterial action ; also it may have helped to precipitate certain soluble protein material. It was noticed that samples of freshly filtered whey from the iron drums, on standing overnight, gave a gelatinous precipitate which settled to the bottom ; this did not occur in whey from the other samples. Thus in the iron drums there would be less soluble protein in the whey and the increase in nitrogen noted would be due to simpler degradation products, whereas the more marked increase of nitrogen and total solids in the whey from W and from the second series, would be due partly to some major portions of the casein molecule becoming dissolved. When the last samples were analysed, after a storage of 159 days, the corrected whey nitrogen figures for the second series had a range of 0.15 to 0.20 per cent., while in the first series after 208 days storage, W was 0.19 per cent., but the samples from the iron drums had a lower and narrower range of 0.12 to 0.15 per cent. These figures are more significant than the trend shown by the total solids figures.

EFFECT OF VARIATIONS

The results of the whey analyses show clearly how certain variations affected the process.

(a) *The use of starter*, i.e., of different types of lactic-acid-producing bacteria, did not appear to vary the process appreciably, but one or two points are worth commenting upon. The drums G and H, L and M, and the cask W were inoculated with *L. bulgaricus* in addition to cheese starter. As compared with G the lactose fermentation in H, sunk underground, at first took place more slowly (as it did in the other sunken drums), but after about a month the lactose values for H became lower than for G, indicating that it was being more quickly used up. This was accompanied by slightly lower total solids and nitrogen values in H, and this tendency persisted till the end of the storage period Neither L nor M were sunk, but the *bulgaricus* inoculation for M was much greater than for L. In M, after about three weeks the lactose was persistently lower than in L. A few weeks after the last addition of buttermilk the nitrogen values for M were lower than for L, and this difference persisted to the end of the storage period. Apart from E to which no buttermilk had been added on 5th November, the only drums in which the lactose content was zero on 5th December were L and M. Possibly this quicker fermentation in L and M may have been due to the *bulgaricus* culture, and the much heavier inoculation of M may have accounted for its lactose being used up more quickly than that of L. The results in W were different from all the remaining drums, partly on account of temperature effects, and partly owing to the absence of iron salts. Whether the *bulgaricus* culture also contributed to the differences is doubtful. In the second series however, the inoculation of S and T, and V with *bulgaricus* did not produce any similar effects, so that the use of *bulgaricus* was probably not the real reason for the effects discussed above in the first series. The use of *L. acidophilus* for X, Y and Z in the second series apparently did not modify the process. Both cultures of lactobacilli which were used grow best at warm temperatures (30 to 37°c.) and on that account may have been less suitable than lactic streptococci. If the controls in both series had not soured readily due to adventitious inoculation derived from cheese whey, they might have shown more clearly the effect of lack of starter addition.

(b) *Temperature* variation produced appreciable differences in several cases. This was noticed early in the slower souring of the buttermilk in the sunken drums of the first series; also the curd in these drums was not so firm at the end of the first week, as a result of slower gas formation. Thus the lactose in these drums lasted longer, as shown in Table IV, where the values for B and F were respectively higher than for A and E. With higher lactose values, higher total solids values were also obtained. This effect of lower temperature was noticeable in H as compared with G for the first month. Later results for this pair were, however, slightly anomalous due to some undisclosed reason.

On sunny days the cask W was cooler than the iron drums so that whey samples from W gave higher lactose and total solids values than the drums above the ground. Again in the second series the vat V was at first shaded by an army hut, and later a screen was placed along its sunny side to check the warping of the wood which was giving rise to leakage. As a result the temperature of the large bulk in the vat did not rise as much as in the casks, so that after 5th December whey from the vat tended to be higher in lactose than from the casks, with a similar though less marked tendency in total solids content. A combination of low temperatures with insufficient milk-souring bacteria at the beginning of the process might however enable undesirable types of bacteria to predominate, and spoil the process.

(c) *Frequency of buttermilk addition,* was observed to produce marked differences. No buttermilk was added to E and F on 5th November, with the result that whey from this pair on 12th November had a lower lactose content than all the rest, while the total solids and lactose for E continued to be lower than all the rest. However, F had a higher total solids and lactose than all except B; this would be due to the fact that larger volumes of whey were removed from E and F on 12th November, and so larger volumes of buttermilk added to replace it, as compared with the remaining drums. The cooler temperature of F underground checked fermentation of the lactose which by contrast took place in E very rapidly. Similar contrasts were noticeable in the total solids and lactose contents of whey samples from E and F on the 5th and 12th November when F continued to have higher lactose and total solids values.

In the *second* series, to observe the effect of longer time between buttermilk additions, none was added to P, R, T and Z on two occasions, 12th and 31st December. In these casks the lactose values of R, T and Z were slightly lower than the corresponding cask of each pair when the whey was analysed a week later (18th December), but samples from the casks P, R, T and Z taken for analysis on 29th gave distinctly higher values for both total solids and lactose as compared respectively with O, Q, S and Y. This was apparently due to the larger bulk of buttermilk which could be added to these casks on the 18th, with the result that the lactose concentration in the whey was raised higher, and so took longer to be fermented. Along with this there was a tendency noticed in the pairs of whey samples of 29th for those with higher lactose to have slightly lower nitrogen (except Z). That difference probably indicated more complete precipitation of the curd. After the final addition of buttermilk on 10th January, further whey samples were not analysed soon enough to enable the repetition to be noted of this effect on the lactose figures, but on 4th February and 4th March in the case of P, R and T, both total solids and nitrogen figures were distinctly lower than those respectively of O, Q and S. The corresponding values for Z were only slightly higher than for Y. These results suggest that additions of buttermilk should be made at longer rather than shorter intervals, in order to keep the curd as long as possible in whey containing lactose, and to enable the curd to separate more completely from the whey.

RESULTS OF CURD ANALYSES

Samples of the curd were analysed at intervals chiefly for total solids and total nitrogen. These determinations were done in one or two cases directly on the wet curd ; in other cases the surplus whey was first drained off, sometimes by pouring away the supernatant liquid, but latterly by placing the curd in a large filter paper (fluted folding) in a funnel. The moisture content, and its reciprocal, the total solids, therefore varied somewhat from batch to batch of samples, and as chloride determinations were not done, adjustments to the results were not possible.

(a) In the *first* series, samples taken on 5/11/45, a few days after the curd was first broken up, yielded total solids values ranging from 12.5 to 13.8 per cent. Well-drained samples taken a week later, 12/11/45, yielded values of about 16 to 18 per cent. Six weeks later, 29/12/45, samples from which the whey had been merely poured off were mostly in the range of 9 to 10 per cent. The last batch of samples, 8/5/46, of the undrained semi-liquid curd had values ranging from 10.0 to 11.5, average 11.0 per cent. When the curd was well drained the total solids values ranged from 13 to 18 per cent., the average for the samples of 8/5/46 being 16.0 per cent. Total nitrogen values on drained curd ranged from 1.2 to 1.8 per cent., so that using the factor 6.38, protein values ranged from 7.6 to 11.5 per cent. None of these results showed any definite trends, but on calculating the nitrogen to total solids ratio as a percentage, figures in the vicinity of 10 were usually obtained. This showed that, despite some slight variations, the protein content of the curd itself was remarkably constant throughout the period of storage.

(b) In the *second* series the total solids values of the curd ranged from about 9 to 12 per cent., and in the well-drained curd from 14 to 16 per cent. The undrained curd samples of 8/5/46 had total solids values ranging from 9.2 to 12.4 per cent. (average 11.0), while the same samples well-drained gave values from 14.7 to 16.0 per cent. (average 15.4). Total nitrogen values obtained on various occasions ranged from 0.9 to 1.1 per cent. for the undrained curd and from 1.4 to 1.8 for the filtered curd. These figures were similar to those of the first series and the same total nitrogen to total solids ratio figures in the vicinity of 10 were obtained with rather less variation. The ratio figures were practically the same for both drained and undrained curd. These results therefore, indicated little change in the composition of the curd and served as a check on the preservation process and also as an indication of the feeding value of the curd, based on its protein content.

In the *second* series, before the curd was first broken the gas underneath pressed the curd into the narrowing opening of each of the casks, and this compacting effect, together with the warm sun and wind, produced a much drier curd. Samples taken from three casks before breaking up the curd to release the gas gave total solids figures around

21 per cent., while one was as high as 29 per cent., but a similar sample from the large vat was just under 17 per cent. These results make clear the saving in storage space which might be achieved if instead of sinking the curd to store it under the whey, it were drained of surplus whey and stored in suitable air-tight containers.

Certain chemical properties of the curd may be deduced from chloride determinations which were done upon only one batch of samples, including both the first and second series. The curd for the analyses had been drained by filtering and the corresponding whey samples were also analysed. At first sight no relation could be traced between the chloride content of the curd and that of the corresponding whey. But on expressing the chloride as a percentage of the water present in each case a fairly definite correlation appeared. This was made evident by calculating the ratio of the chloride in the curd water to the chloride in the whey water. Out of samples from 21 different containers, this ratio in the case of no less than 12 lay between 1.16 and 1.19, with an average of 1.18. For the remaining 9 containers the ratio in 2 cases was below 1.0, while in the 7 other cases it ranged from 1.08 to 1.26 with an average of 1.19. The fact that the chloride ion concentration in the water associated with the curd was higher than it wasin the whey water was probably due to a Donnan membrane effect. If this were the case the ratio might be altered by changes in the physico-chemical state of the curd, particularly its degree of hydration, and this in turn might alter as breakdown from larger to smaller molecules took place. Unfortunately chloride determinations were not done regularly on the curd so that this possibility could not be further studied.

In the undrained curd, which in practice, would be the product used for feeding, about 89 per cent. was water when the last samples were taken, and of the 11 per cent. total solids, fully 6 per cent. was protein. The remaining 4 per cent. consisted of ash, salts of such organic acids as lactic acid, etc., and a small amount of fat, usually less than 1 per cent.

From the results of the chemical analyses for total solids and for nitrogen some idea can be obtained of the concentration effected by the process. The original buttermilk had a nitrogen content of about 0.4 per cent. Results obtained from undrained curd of the first series sampled on 29/12/45, gave an average total solids value of 9.4 and this curd was found to have its nitrogen about 10.2 per cent. of its total solids. Thus the nitrogen content of the curd itself would be about 0.96 per cent. The concentration figure is therefore 0.96/0.4, i.e., 2.4. Similar calculations from results obtained towards the end of the storage period gave a rather higher figure, which approached figures obtained likewise from the second series, 1.12/0.4, i.e., 2.8.

Starting from the original buttermilk total solids figure of about 7.5 per cent., this becomes only 11 per cent. in the final undrained curd; but if allowance is made for the loss of lactose, about 3.4 per cent., the original buttermilk solids drop to 4.1 (i.e., 7.5 minus 3.4). Thus the concentration figure is obtained by dividing 11 by 4.1 giving the result, 2.7, which is only a little lower than the figure derived from the nitrogen analyses.

However according to conditions some variations may occur, so the concentration or reduction in volume effected by the process can be reckoned to be rather less than three times. After long storage, evaporation may cause the concentration to approach or even exceed three times. This effect was made clear during the Featherston trials by chloride determinations on the whey, which rose from below 0.07 to about 0.09, with odd samples above 0.1 per cent.

GENERAL DISCUSSION

During these trials curd was prepared and stored in good condition in various containers for periods of 6 to 8 months. By chemical analysis of the buttermilk, whey, and curd, information was obtained about the changes which occurred during the storage process. On account of other work in hand it proved impossible to do as much analytical work as might have been useful, nor was it possible to work up the results and study them in detail during the course of the trials. Consequently some factors which might profitably have been studied by fuller analysis were overlooked. During the progress of the trials notes were made of various details such as the amount of curd floating, the variation in gas condition of the whey, differences in colour and cloudiness of the whey during the early stages while buttermilk was still being added. The possible correlation of such points with later developments did not eventuate. In planning the trials an important consideration was to vary the conditions as much as possible to see if some were preferable to others. In retrospect it appears that more chemical information might have been obtained if fewer containers had been used so that more frequent sampling for analysis could have been undertaken. This would have provided a more complete chemical picture of the progress of the changes.

On buttermilk samples, lactometer readings were done usually the same day as they were taken, but if it had been possible to do also total solids, lactose, etc., the same day instead of the following day that would have enabled the amount of added water to be determined fairly accurately, and thus differences in the composition of the buttermilk on different occasions might have been explained. Fat determinations on buttermilk would have enabled Richmond's formula (2) for total solids, and Udy's (3) for added water to have been applied. The weighted average figure for chlorides is more accurate for the first series than for the second series in which some of the earlier samples of buttermilk were not used. However if the basic chloride figure were varied slightly that would not affect appreciably the general trend of the results.

Throughout the trials the whey samples were very fully analysed, but more frequent total nitrogen and formol determinations in the early stages might have been useful. In addition, total protein nitrogen as distinct from total nitrogen determinations would have helped to show how much of the nitrogen was due to casein which had not aggregated upon the curd, especially in the earlier stages while buttermilk was still being added. Total protein determinations would also have been useful to indicate the trend of casein decomposition and to supplement information yielded by formol titrations. In some cases slight differences were noticeable in the composition of the whey which was separated by pouring from the curd and that obtained by filtration but it was not possible to investigate these differences, which were chiefly in the nitrogen content.

So long as the mixture of the curd and whey remained at a fairly low pH, as determined on the whey, the need for detailed analysis of the curd seemed to be not quite so pressing. The curd analyses were therefore confined chiefly to total solids and nitrogen determinations, and these served to indicate the constancy of composition of the curd, as well as its feeding value. As already pointed out chloride determinations on the curd might have provided some useful data to indicate changes in its physico-chemical condition. To provide fuller information about the feeding value of the stored product, fuller analysis would have been useful especially on account of the gap between total solids and total protein content. A few determinations showed that this gap was partly filled by the presence of fat (about 1 per cent.), ash (about 1 per cent.), and lactic and other organic acids. A series of determinations would have helped to provide more data about these constituents, which were however secondary to the main feeding value of curd derived from its protein content.

Provided a sufficient inoculation of lactic-acid-producing bacteria is present originally, a high acidity, (pH 4.2 to 4.4), can be obtained fairly rapidly so as to precipitate and coagulate the casein into curd. Apart from the formation of the curd, the souring also plays an important part in the storage because when the material is sufficiently acid the multiplication of a variety of bacteria is checked, and putrefaction cannot so readily occur.

It is a well-established principle that in the presence of a fermentable carbohydrate, proteolytic bacteria use this carbohydrate in preference to attacking protein material (4), (5). In the present investigations this principle appeared to apply for there was little decomposition of the curd while lactose was present, and after the lactose was all used up, the curd dissolved very slowly, as shown by determination of both total nitrogen and formol values in the whey. These results showed that it was desirable to maintain the lactose content as long as possible and this could be done in two ways; firstly by spacing out the additions of buttermilk at longer intervals (10 to 15 days) rather than shorter (5 to 10 days); secondly the slower fermentation of the lactose in the sunken drums showed that a lower storage temperature was preferable. After the lactose was used up there was practically no tendency for the curd to rise to the surface of the whey, and at that stage another important factor operated in conjunction with the acidity, namely the exclusion of air which was effected by keeping the curd covered with whey. Just as in making ensilage, it was important to exclude air so as to check the multiplication of various types of bacteria which were capable of growing and decomposing or putrefying the curd.

The acid conditions in the curd would no doubt favour the growth of very large numbers of harmless lactobacilli (e.g., *Streptobacterium casei*) which are common in cheese. Apart from these the principal types of organisms likely to grow are yeast and moulds. The former are less important than the latter which usually produce appreciable amounts of ammonia and similar substances which are capable of neutralizing the acidity. If this should occur in a confined spot where the effect is localized the pH may rise and enable other bacteria present to develop so that decomposition may soon proceed rapidly; but any mould spores which chance to be blown on to the surface layer of whey will sooner or later become submerged due to air currents so that mould growth is thereby checked. This is one advantage of not having these containers provided with covers. Towards the end of the trials when covers were required on the sunken drums to prevent the access of farm animals, a film of mould growth developed here and there on the surface of the whey.

If the storage of the curd were attempted by draining the whey from it and placing it in a cask or drum, special care would need to be taken to use an air-tight container and to press the curd well down so as to exclude air. A tight fitting lid would also be necessary to limit the access of air to the top as much as possible, with the object of checking the growth of mould and proteolytic bacteria.

The results showed that the preservation of curd in many respects followed the traditional process of cheese-making. The final product was of course very different from cheese made in a factory where the curd was removed from the whey, drained and pressed but the underlying bacteriological and chemical changes were essentially the same. In both cases acidity was developed to produce the curd and check the growth of undesirable bacteria, which were also suppressed by the exclusion of air.

Towards the end of the storage period the rising nitrogen content of the whey revealed a slight tendency for the curd to decompose. This would normally be checked with the advent of cooler weather, and longer storage would not often be needed. Although some variations were noticeable in the results of the whey analyses, especially during the earlier stages, after six to eight months' storage no significant differences were noticeable in the properties of the curd in the different containers. From this it may be deduced that considerable latitude is permissable in the preparation. Nevertheless, to secure the maximum efficiency of storage, due attention should should be given to certain points which have already been emphasized, e.g., liberal inoculation with an active cheese starter culture to initiate rapid souring ; avoidance of warm temperatures by shading the containers from the sun, and addition of buttermilk or skim milk after longer rather than shorter intervals. These and various other practical points (including food values and economic aspects) have been described in detail in an illustrated article (1).

These investigations were sponsored by the National Pig Industry Council, which provided a small grant from its funds towards the expenses. Much valuable assistance with many practical details was rendered by Mr. H. M. Peirson, Superintendent of the Pig Industry. Besides other assistance which has been acknowledged in the Bulletin (1), mention should be made here of help given by Mrs. D. R. Perrin with some of the chemical analyses, and by Mr. A. L. Bryant in preparing the graphs.

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APPENDIX OF ANALYTICAL METHODS

The analytical methods which have been used are described separately in this Appendix so as to avoid confusion with the principal subject matter.

SPECIFIC GRAVITY : This determination was carried out on all buttermilk samples, except those which had became sour, by adjusting the temperature to $60 \pm \frac{1}{2}$°F. and using a finely graduated lactometer.

ACIDITY : (a) *Titration*. To indicate the progress of souring 9 ml. of buttermilk or whey was titrated with 0.1N. sodium hydroxide using 1 ml. of 1 per cent. phenolphthalein per sample, and the results were recorded conventionally as "per cent. lactic acid". Satisfactory results were obtained in the early stages of souring, but with very sour samples from the casks or drums, the presence of curd particles of varying size gave unreliable results. Clear whey could be easily titrated but when more or less finely divided curd was dispersed in the whey, the results were erratic.

(b) *pH*. To find the pH of whey samples, 0.5 ml. of 0.04 per cent. bromo-cresol-green indicator was added to 10 ml. portions and the colour matched in a Lovibond comparator.

TOTAL SOLIDS: Into previously dried porcelain dishes about 3 to 5 g. of curd or 10 g. of buttermilk (pipetted) were quickly weighed. Light aluminium dishes were preferred for 10 g. whey samples (also pipetted) The dishes were heated for at least three hours in a steam oven, then cooled and weighed, followed by two heatings of at least one hour each in the oven, with subsequent weighings. Owing to the acid condition of the material further heating gave slight further loss in weight due to volatile fatty acids, etc., being driven off. Occasional trials of longer heating were made, but the effect on the results of further slight losses in weight were hardly significant. As the dry material was very hygroscopic, speed was necessary with the final weighings, and too many dishes could not be placed in one desiccator. The results have been expressed as grams of dry matter per 100 g. of sample.

Determinations were carried out as soon as possible after collecting the sample, lest storage, particularly for curds, should lead to the production of volatile products, thereby lowering the total solids value. When weighing curd samples thorough mixing was necessary to ensure agreement between the duplicates.

CHLORIDES: According to Volhard's method, to 25 ml. of whey or buttermilk in a 300 ml. conical flask, were added 10 ml. of accurately standardized tenth-normal silver nitrate, followed by 10 ml of concentrated nitric acid (commercial acid was found satisfactory if chloride free). The flasks were boiled gently on a hot plate until the liquid cleared. To determine the unprecipitated silver, the resultant solution, after diluting to fully 100 ml. was titrated with approximately tenth-normal potassium thiocyanate solution, using iron-alum as indicator. For each batch of samples, blanks were done in order to standardize the potassium thiocyanate and as a check on the purity of the nitric acid. The results were expressed as grams of chloride (Cl^- ion) per 100 g. of sample.

Later, when insufficient whey was available, determinations were done on the mixture resulting from the formol titration, containing 10 ml. of whey. In this case 5 ml. tenth-normal silver nitrate and 5 ml. of nitric acid were added. To avoid excessive frothing due to the formalin, these samples were warmed gently in a water-bath. The presence of the formalin did not appear to affect the results obtained as compared with those given by the usual method with 25 ml. of whey. For dealing with smaller amounts of whey, a finely graduated burette and more dilute standard silver nitrate and thiocyanate solutions were preferable.

On some of the later batches of curd a chloride determination was done on 10 g. of curd using 5 ml. of tenth-normal silver nitrate and 20 ml. of nitric acid. Rather longer heating was required to complete the digestion, after which the solution was diluted and titrated in the usual way.

LACTOSE: Determinations were done on buttermilks and on whey samples obtained at intervals until all the lactose had been fermented. For this purpose the method of Lane and Eynon (1) was used as modified by McDowell (2). This method depends upon the treatment of a precise amount of Fehling's solution with a portion of the milk or whey sample and subsequent titration of the unreduced copper with standard lactose solution. The quantity of lactose solution equivalent to the Fehling's solution may be determined by a blank titration. Hence may be obtained the amount of standard lactose solution equivalent to the lactose in the milk or whey, and therefore the percentage of lactose in the sample. There are two steps to the determination; first, an approximate titration to give a preliminary indication and secondly an accurate titration to give the precise result. Fat and protein need not be removed as in other methods provided the sample is sufficiently diluted with distilled water. Four solutions are required, and equal volumes of numbers 1 and 2 are combined to form Fehling's solution, taking care to pipette No. 1 accurately.

1. Copper Sulphate:—Dissolve 34.639 g. of Analar $CuSO_4.5\ H_2O$ in 500 ml. of distilled water. This solution is the standard and must be accurately made up in a standard flask.

2. Alkaline Tartrate:—Dissolve 173 g. of pure sodium potassium tartrate, and 51.6 g. of NaOH in 500 ml. distilled water. The equivalent amount of bi-potassium tartrate may be used. It is convenient to use approximate 50 per cent. NaOH solution as this is carbonate-free and its strength may be estimated by titrating.

3. Lactose, 0.5 per cent:—Dissolve 2.50 g. of lactose monohydrate in 500 ml. of water in a standard flask. This standard solution is best made up as required for it tends to grow moulds.

4. Methylene Blue, 1 per cent.:—Dissolve 0.1 g. in 10 ml. water, by warming on a water bath. It is preferable for this solution to be fairly fresh.

With an accurate pipette, 5 ml. of solution 1 were measured into a 250 or 300 ml. conical flask and then 5 ml. of solution 2 were added by pipette, or burette (but not glass tap). To the mixture, 2 ml. of the sample were accurately pipetted (less or more according to the lactose content). After adding 10 ml. of distilled water, the mixture was heated quickly till it boiled. After boiling for two minutes, if reduction was fairly complete, 2 drops of methylene blue were added. If however, the colour indicated that reduction was incomplete, 2 to 3 ml. of lactose solution were added, followed by 2 drops of methylene blue. After 30 seconds more boiling, the flask on stand and burner were moved to underneath the burette, whence lactose solution was gradually run in, with 10 seconds boiling between each addition, until the methylene blue decolourized and the liquid became bright red.

As the methylene blue is an internal indicator, the colour change is sudden, from deep purple to bright brick red and is usually completed with one drop of lactose solution. In all, the solution should not be boiling for more than five minutes. With experience, the analyst could tell approximately how much reduction had taken place, by the appearance of the solution when it was first boiled. The flask should not be heated directly under the burette for longer than is absolutely necessary, especially in the final titration.

For the accurate final titration, the same quantities of solutions and whey or buttermilk were pipetted out, but before boiling, the standard lactose solution was run in to within 0.5 ml. of the amount already determined.

The mixture was boiled (away from the burette) for two minutes, indicator added, and then the remaining lactose solution was added drop by drop while being boiled under the burette, until the end point was reached.

The results were calculated as follows: - If A were the volume (ml) of 0.5 per cent. lactose required for 10 ml. of Fehling's solution and B the volume required to complete reduction after adding X ml. of sample, then 1 ml of sample equalled $\frac{A - B}{X}$ ml of 0.5 per cent lactose solution Therefore the lactose in 100 ml. of sample was $\frac{A - B}{X} \times 0.5$ As the standard solutions were all made up from lactose monohydrate the results were expressed as grams of lactose monohydrate per 100 ml

NITROGEN: (a) For *Total Nitrogen* the usual Kjeldahl method was at first used. In this 3 to 5 g. of well-mixed curd were digested with 25 ml. of concentrated sulphuric acid and 5 g of anhydrous sodium sulphate, using 1 ml of 5 per cent. copper sulphate solution as catalyst. To ensure complete conversion to ammonia, care was taken to continue heating for fully an hour after the liquid cleared After diluting sufficiently the clear digest was made alkaline with about 60 ml of approximately 50 per cent. sodium hydroxide solution, and the ammonia distilled into 0.1 N. sulphuric acid. The excess acid was titrated with 0.1N caustic soda, using methyl red—methylene blue indicator. Blank determinations were done with each batch, and the results expressed as grams of nitrogen per 100 g. of curd.

Total nitrogen was done on the earliest batch of whey samples by the above macro-method using 10 ml of whey, but, as too much time was needed to deal with large numbers of samples, a micro-method was used. The method adopted employed a slightly modified digestion mixture and the Parnas-Wagner Kjeldahl steam distillation outfit similar to that described by Clark (3) Either 1 or 2 ml. of whey was digested with 1.5 ml. of concentrated sulphuric acid and 0.5 g. of sodium sulphate, with 40 mg. of mercuric oxide as a catalyst. The clear digest was diluted, transferred to the distillation outfit, and made alkaline with a solution containing 40 per cent. sodium hydroxide and 5 per cent. sodium thiosulphate. The ammonia was then distilled into 2 ml. of 4 per cent. boric acid solution and the solution titrated with 0.02 N. hydrochloric acid using methyl red—methylene blue indicator. The acid was diluted as required, from 0.1 N. hydrochloric, which was checked by the usual silver nitrate method The results were expressed as grams of nitrogen per 100 g. of whey.

(b) *Protein Nitrogen.* In order to get an indication of the amount of protein which would be available as curd, the first samples of buttermilk were analysed for casein and total protein according to the method of Moir (4). Briefly the method is as follows:—The casein was precipitated from 10 ml. of buttermilk diluted with water, by the addition of 1.5 ml. of 10 per cent. acetic acid followed by 4.5 ml. of 0.25 N. sodium acetate; after filtering and washing, the nitrogen content of the precipitate was determined in the usual manner. The total protein was similarly precipitated from 10 ml. of buttermilk with 20 ml. of 10 per cent. trichloracetic acid, and the mixture was warmed to 50°c. for a few minutes before filtering. There was insufficient time to carry out protein partitions on any of the later buttermilk samples nor upon the whey samples.

(c) *Amino-Nitrogen.* To indicate the extent of protein decomposition, Sorensen's "Formol" method was applied to 10 ml. portions of the whey. After titrating with 0.1 N. sodium hydroxide to the first definite shade of pink (with 1 ml. of 1 per cent.

phenolphthalein as indicator), 2 ml. of approximately 33 per cent. " water white " formalin were added and the solution re-titrated to the same shade of pink. From the result was subtracted a blank done on 2 ml. of the formalin diluted to the same extent with distilled water. The first titration (without formalin) gave the acidity of the whey, but these values were somewhat erratic

The so-called formol figures might have been converted to the equivalent percentage of amino-nitrogen by using a suitable factor, but in the present work it sufficed to use formol titration to total nitrogen ratio. The figures so obtained were quite arbitrary but indicated the extent to which the total soluble nitrogen was becoming decomposed.

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ADDENDUM

(Received for publication, 1st June, 1948)

Since the foregoing was written we have had occasion to examine samples of curd which had not been successfully stored. On a farm in North Auckland 12 forty-four gallon drums were used to store curd prepared from skim milk. Seven of these were successful, but the other five began to ferment and putrefy after froth from skim milk had been added to them. In the following table are shown the most important of the analytical results. The wet curd from the bottom of the containers was drained by filtering, so that the curd portion and the whey portion could be analysed separately. Sample A was from a drum showing considerable putrefaction, sample B was moderately affected, while sample C was from a drum which appeared to be normal. For the purpose of comparison the results have been adjusted to the average chloride content (.081 per cent.).

	A	B	C
pH of Whey	5.5	4.25	4.0
Acidity Titration (ml. 0.1 N. NaOH for 10 ml. Whey)	7.2	25.2	33.0
Whey Nitrogen (g. N per 100 g. Whey) ...	0.584	0.407	0.382
Formol Titration (ml. 0.1 N. NaOH for 10 ml Whey)	21.0	9.4	6.04
Formol to Nitrogen Ratio, Whey	36.0	23.1	15.8
Percentage of Total Nitrogen in the Whey ...	30.0	21.5	16.9
Curd Brine to Whey Brine, Ratio	1.08	1.25	1.31
Curd Total Solids	18.1	15.9	15.7

The results in the table show a distinct difference and graduation from A to C. The values obtained from the ratios of curd brine to whey brine confirm the rather scanty evidence about this point which was discussed in the section on curd analyses. Decomposition of the curd reduces the amount of chloride which can be associated with it. The use of buttermilk for the Featherston trials may account for the ratio there being about 1.2 as compared with 1.3 given by curd from drum C, prepared from skim milk.

The total solids of the whey varied from 5.1 to 5.7 per cent. The nitrogen figure for the curd was between 1.73 and 1.89 per cent. while the percentage of nitrogen in the total solids of the curd was 9.5 in sample A, and in the vicinity of 11.5 for the other samples. Sample A was grey in colour while the others were whitish. The dark colour was probably due to the presence of ferrous sulphide formed from the iron dissolved from the drums and hydrogen sulphide from the breakdown of the protein A positive test for sulphide was obtained from sample A, but not from the others.

The reason for the failure of A and B is as follows : —As the foam would resist attempts to mix it with the rest of the contents of the drum, the required acidity would not be quickly developed. The lack of acidity, together with the plentiful supply of air in the foam, would permit large numbers of different types of bacteria to develop and attack the protein. In addition, when the putrefactive bacteria became sufficiently well established they would then produce less acid conditions in the rest of the drum and so allow the putrefactive process to continue throughout the drum. This has obviously happened in sample A, and to a much less extent in sample B.

THE EFFECT OF SHEEP DROPPINGS ON YIELD, BOTANICAL COMPOSITION, AND CHEMICAL COMPOSITION OF PASTURE

II. RESULTS FOR THE YEARS 1942-1944 AND FINAL SUMMARY OF THE TRIAL

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(Continued from Volume XXIV, No. 1A, page 61A)

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Summary

Final results of a trial at Palmerston North to measure the effects of sheep dung and urine on pasture growth and composition are presented. Preliminary data were given in a previous paper and this report covers the final three years of the trial

Grazing without return of dung or urine resulted in an average production of 10,000 lb. of dry matter per acre per annum This was largely the result of sustained growth of the clovers which were adequately supplied with added phosphate and lime, but the growth of associated grasses was quite good.

Return of urine resulted in an increase in total dry matter production to the extent of 15 per cent over no return, with a much higher proportion of grass in the sward.

Return of dung gave a similar (18 per cent) increase in total dry matter production There was a progressive increase in proportion of grass in the sward following the high preponderance of clovers in the early stages of the trial.

Return of both dung and urine gave an increase of 33 per cent. over the period The grass dominance which developed in the early stages of the trial increased during the final two years.

Chemical compositions of herbage, dung and urine are presented together with balances for nitrogen, potassium, phosphorus and calcium in feed and excrement The balances show reasonable agreement except in the case of nitrogen The possible sources of error are discussed

INTRODUCTION

THIS paper presents the final results of the trial previously described in a preliminary report by two of the present authors (1).

In that paper the objects, layout, and measurement techniques were fully detailed, the literature was discussed, and the results of the first 18 months of the trial were presented. Subsequent minor alterations in the conduct of the trial are discussed in the appropriate sections of this paper.

Briefly the trial was designed to measure changes in sward composition and productivity of a mixed pasture grazed by wether sheep under the following systems of management:

(a) Sheep grazing with natural return of droppings (F.R.)*
(b) Sheep grazing with no return of droppings (N.R.)*
(c) Sheep grazing with return of urine only (U.R.)*
(d) Sheep grazing with return of dung only (D.R.)*

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* The initials F.R., N.R., U.R. and D.R. are used throughout the text in referring to the four treatments under trial

The pasture was sown in March, 1940, with a complex mixture of pedigree grasses and clovers. Superphosphate at 4 cwt. per acre was applied at sowing down and annually thereafter in April. Carbonate of lime at 10 cwt. per acre was applied at the same time in alternate years. Collections of dung and urine were made by the use of the harness and containers previously described (2).

Each treatment consisted of a pair of one tenth ($\frac{1}{10}$) acre paddocks, separately fenced, and so arranged that the block as a whole consisted of two comparable groups of four paddocks each.

When the pastures were ready for grazing the sheep were put on to one set of paddocks and measurements made within this group. After grazing was completed the sheep were transferred to the similar treatments in the duplicate set of paddocks. Depending on growth conditions, the sheep were then either returned to their first set of paddocks or else removed from the whole area to await the next grazing. It will therefore be appreciated that although each treatment consisted of a pair of separate paddocks, these two are not true duplicates since they were never grazed simultaneously. The small differences which would result should, however, be evened out over several grazings, and for the four year period covered in the trial, the two paddocks of each pair may justifiably be considered as valid duplicates.

For the first 2-3 years of the trial, grazings were carried out when the growth reached 2 to 3 inches and this was grazed down to about 1 inch in height. Due largely to shortage of collection gear, only small numbers of sheep were used at each grazing. Thus the management of the pastures was such as to produce good ryegrass-white clover swards, and since the sheep were on the paddocks for a large proportion of the time, total collections of dung and urine for the two paddocks of each treatment could be expected to be reasonably complete.

Later on, ample collection gear became available, but because of staff shortages and overlapping the work with other trials, it became necessary to reduce the number of grazings so that the pastures were generally 4 to 6 inches in length at the beginning of each grazing. This change in management favoured the growth of cocksfoot which came into prominence in all treatments towards the end of the trial. On the other hand the more lax grazing management was no doubt detrimental to the full development of the ryegrass. During this period larger numbers of sheep were used for shorter times on the paddocks ; between grazings they were held with the general Station sheep on feed that was probably not quite as plentiful. The dung and urine collected during grazing of the paddocks were probably not as true a reflection of the feed ingested as were the earlier collections. Also with the longer grass growth, some feed may have been wasted by trampling and fouling. The general overall effects of the treatments do not appear to have been significantly affected by the modification ; they are, however, probably the main reason for the poorer nutrient balances obtained in the last two years.

EXPERIMENTAL TECHNIQUE AND FERTILIZERS

Following the original dressings of 10 cwt. of lime and 4 cwt. of superphosphate per acre at the time of sowing and a further dressing with 4 cwt. of superphosphate in August 1941, the whole area was topdressed by hand as follows :—

April, 1942 :—Superphosphate at 4 cwt. per acre plus carbonate of lime at 10 cwt. per acre.
April, 1943 :—Superphosphate at 4 cwt. per acre. No lime.
April, 1944 :—Superphosphate at 4 cwt. per acre plus carbonate of lime at 10 cwt. per acre.

Pasture yields were determined throughout by the use of protecting cages as previously detailed. After the sheep had been removed from the paddock, the protected herbage was clipped, by hand, down to the same height as the grazed herbage.

Samples for botanical and chemical analyses were obtained by cutting 60-80 individual areas (each of approximately 30 square inches) per paddock prior to each grazing. These samples were then mixed and sub-sampled for analysis. Botanical analysis was made by direct dissection of the fresh herbage, the separations then being oven-dried and the composition determined on the dry matter basis. Chemical compositions of the herbage were determined by the methods previously described (1). Collections of dung and urine and their sampling continued as previously described, but in addition, complete collections were made from two sheep on each treatment for the 1941-42 season, to give a check on the chemical composition of the excrements for the different treatments, as well as to obtain a balance between ingestion and excretions for that period. The materials thus collected were measured, sampled and then returned to their appropriate paddocks e.g. on the full return treatment, two sheep only were collected from, and all collections were returned, while on the urine return treatment the dung was collected from all sheep, but in addition two sheep were harnessed for urine collection and this urine was sampled and then returned to its paddock.

The chemical analyses of the dung and urine followed the methods earlier described (1), but due to the very small amounts of calcium and phosphorus found previously in the urine samples, these constituents were not determined in the later stages of the trial.

For the chemical analysis of the herbage, the dung and the urine, the samples were bulked in proportion to the yields for the several measurements made within the periods shown, and the analysis made gives a direct figure for each period. Each period shown tallies with completed grazings of the experiment as a whole. The botanical analyses were, however, of necessity made on each sample as cut fresh after each paddock grazing, and then the dry weight percentages of the species present were determined. The calculations of the botanical compositions as shown for the periods covered by the chemical compositions, have been made by calculating the total weight of the various species for each particular paddock within that period. After addition of the two sets of total species yields for two paddocks of each treatment, these were then calculated as percentages of the total dry matter yields for the four treatments, and thus the botanical and chemical analyses are on the same sampling basis. No adjustment could, of course, be made for the final laboratory moisture figure, but as this is very small in itself, no significant error is introduced.

RESULTS

The data secured have been summarized into a series of tables similar to those in the earlier paper. These are shown in detail for the period covered by this report (22nd July, 1941, to 30th May, 1944) but the totals quoted include also the results from 22nd July, 1940 to 21st July, 1941, and quoted in the earlier paper (1).

Table I sets out the monthly mean figures for soil temperatures at the 1 ft. level and the total monthly precipitations, together with departures from the previous ten-year averages. The relatively small overall range in soil temperatures (46-67°F.) together with the fairly even spread of rainfall, give a good picture of the excellent all-the-year-round growth conditions which normally occur at Palmerston North. The only outstanding feature of the weather for the period of the trial was the very hot autumn of 1944. At this time, high soil temperatures coincided with very low rainfall, and these conditions were reflected in much reduced pasture yields and a marked change in chemical composition.

TABLE I. METEOROLOGICAL DATA RECORDED AT GRASSLANDS DIVISION, PALMERSTON NORTH
(Station situated 10 chains from experimental block)

Month.	Soil Temperature at 1 ft.		Rainfall (Inches).	
	Mean for Month.	Departure from Ten Year Average. 1930-1940.	Total.	Departure from Ten Year Average. 1930-1940.
1941	°F.	°F.		
August	45.0	—1.9	2.95	—0.61
September	50.2	—0.3	2.92	—0.14
October	53.9	—1.0	5.19	+1.95
November	57.6	—1.4	4.59	+1.60
December	62.1	—1.5	4.62	+1.64
1942				
January	62.5	—3.7	3.96	+1.18
February	63.4	—1.1	3.43	+0.50
March	60.7	+0.4	4.47	+1.95
April	58.4	+2.2	1.86	—1.65
May	52.7	+1.1	4.93	+1.40
June	47.0	0.0	0.61	—3.29
July	48.2	+4.4	5.46	+2.71
August	47.8	+0.	2.88	—0.78
September	51.2	+0.7	4.04	+1.02
October	57.6	+2.7	1.93	—1.31
November	59.7	+0.7	3.09	+0.10
December	63.5	+0.1	1.65	—1.33
1943				
January	66.5	+0.3	2.60	—0.23
February	66.0	+1.5	5.75	+1.82
March	63.1	+2.8	1.06	—1.46
April	58.3	+2.1	2.04	—1.47
May	51.8	+0.2	1.33	—2.20
June	49.5	+2.5	6.59	+2.69
July	46.3	+2.5	4.22	+1.47
August	46.5	—0.4	2.63	—0.93
September	50.9	+0.5	5.70	+2.64
October	55.7	+0.8	3.48	+0.24
November	60.8	+1.8	1.97	—1.02
December	66.4	+0.8	1.98	—1.00
1944				
January	67.1	+0.9	1.39	—1.44
February	66.5	+2.0	1.15	—2.78
March	64.6	+4.3	3.51	+0.99
April	59.7	+3.5	3.85	—0.16
May	52.0	+0.4	1.25	—2.28
June	47.4	+0.4	3.11	—0.79
July	46.5	+2.7	1.67	—1.08

In Table II are shown the total dry matter yields as measured for each pair of paddocks within the treatments. Due to the fact that all chemical determinations were done on samples proportionately bulked from both paddocks of each treatment, the figures shown in Table II do not take into account the final moisture content determined in the laboratory analyses. The totals are therefore greater then those shown in subsequent tables by 3 to 5 per cent. Considering that the two paddocks within each treatment are not absolutely comparable because of the different grazing times, the agreement in total annual productions is reasonably good. The appended statistical analysis shows that the treatment differences are highly significant between N.R. and F.R. and that U.R. and D.R. differ significantly from both N.R. and F.R., but with no significant difference in total productions between the U.R. and D.R. treatments themselves. The totals for the first year of the trial (22nd July, 1940, to 21st July, 1941) are included for completeness.

TABLE II. TOTAL DRY MATTER YIELDS OF DUPLICATE PADDOCKS WITHIN TREATMENTS FOR PERIODS SHOWS

(All figures in lb. D.M. per acre before laboratory adjustment for residual moisture)

Treatment.	Date.	Yields of Paddocks.		Treatment.	Date	Yields of Paddocks	
		A	B			A	B
Full Return	22/7/40-21/7/41	14936	13666	Urine Return	22/7/40-21/7/41	12426	13914
	21/7/41-28/5/42	14498	16738		21/7/41-28/5/42	11712	10844
	28/5/42-20/5/43	13596	14343		28/5/42-20/5/43	14680	12414
	20/5/43-30/5/44	13974	15250		20/5/43-30/5/44	12928	11325
	Total for 4 years	57004	59997		Total for 4 years	51746	48497
	Average per year	14251	14999		Average per year	12936	12124
	Mean Annual Yields	14625			Mean Annual Yields	12530	
No Return	22/7/40-21/7/41	13535	10675	Dung Return	22/7/40-21/7/41	13158	12037
	21/7/41-28/5/42	11272	9841		21/7/41-28/5/42	12659	12322
	28/5/42-20/5/43	9659	9575		28/5/42-20/5/43	13074	13226
	20/5/43-30/5/44	10834	11824		20/5/43-30/5/44	14233	12513
	Total for 4 years	45300	41915		Total for 4 years	53124	50098
	Average per year	11325	10479		Average per year	13281	12524
	Mean Annual Yields	10902			Mean Annual Yields	12903	

Analysis of Variance

Total Dry Matter Yields of Duplicate Paddocks within Treatments (Table II)

Source of Variation.	Degrees of Freedom.	Mean Square × 10^{-3}	F.
Blocks	1	13890	
Treatments	3	186742	15.446
Years	3	5326	
Blocks × Treatments	3	12090	
Blocks × Years	3	2678	
Treatments × Years	9	17426	
Blocks × Treatments × Years	9	13009	
Total	31		

Significance of Differences between Treatment Means

	Full Return.	No Return.	Urine Return.	Dung Return
Full Return	—	S.S.	S	S
No Return	—	—	S	S
Urine Return	—	—	—	N.S.

Table III sets out the complete period totals for pasture production and the botanical and chemical compositions determined for those periods. The yields are totals (on a per acre basis) for the duplicate paddocks, and include all cuts actually made within each period.

The seasonal variations in growth can be seen, although the periods are not of equal intervals such as would show this more clearly. The data have, however, been regrouped to show the point more clearly in Table VI. Similarly, variations in botanical compositions can be seen for each period.

TABLE III. YIELDS OF HERBAGE FROM THE TREATMENTS, AND DETAILS OF CHEMICAL AND BOTANICAL ANALYSES AT EACH SAMPLE BULKING DATE

Date.	Dry Matter per Acre in Pounds.	Botanical Composition (per cent. Dry Matter).					Ratio of Grasses to Clovers.	Chemical Analyses (per cent. Dry Matter).				
		Perennial Ryegrass.	Other Grasses.	White Clover.	Red Clover.	Other Species.		Total N.	Soluble Ash.	CaO.	P_2O_5.	K_2O.
A. Full Return												
9/8/41	439	80	4	14	2	—	5.3 : 1	4.26	7.90	0.80	1.11	3.90
19/9/41	2308	68	4	25	T	3	2.9 : 1	4.36	8.59	0.93	1.06	4.11
18/10/41	1441	68	11	20	1	—	3.3 : 1	4.38	10.97	1.14	1.43	4.85
3/12/41	2920	67	10	21	2	—	3.0 : 1	4.33	9.58	1.19	1.08	4.44
19/12/41	1786	51	6	37	6	—	1.3 : 1	4.71	10.07	1.30	1.09	4.46
26/1/42	2067	46	9	41	2	2	1.3 : 1	4.76	10.05	1.39	1.10	4.47
17/2/42	1764	59	4	33	T	4	1.9 : 1	4.52	10.65	1.23	1.06	4.86
16/3/42	1139	52	3	42	T	3	1.3 : 1	4.68	9.67	1.36	0.99	4.56
16/4/42	1098	60	3	37	T	—	1.7 : 1	4.39	10.52	1.11	1.08	4.67
28/5/42	658	59	5	30	T	6	2.1 : 1	4.51	9.93	1.08	0.96	4.56
30/7/42	1346	70	12	18	T	—	4.6 : 1	4.35	9.12	0.94	1.17	4.13
10/9/42	1460	57	20	21	—	2	3.7 : 1	4.27	9.35	1.07	1.16	4.18
23/10/42	2725	68	9	21	T	2	3.7 : 1	4.75	9.92	1.01	1.11	3.60
15/11/42	850	68	20	7	T	5	12.6 : 1	4.40	9.49	1.11	1.02	4.21
19/12/42	2811	61	12	24	T	3	3.0 : 1	4.21	9.40	1.18	0.91	3.91
26/2/43	2736	54	10	28	2	6	2.1 : 1	4.70	9.73	1.26	1.05	3.44
26/3/43	1143	57	7	35	1	T	1.8 : 1	4.35	10.25	0.98	0.93	4.41
20/5/43	900	77	9	14	T	T	6.2 : 1	4.16	9.22	1.04	0.76	3.76
2/9/43	1828	71	21	6	T	2	15.3 : 1	4.28	8.28	0.66	0.94	3.70
5/10/43	1747	68	15	15	—	2	5.6 : 1	5.13	9.68	1.01	1.52	4.30
26/10/43	1537	67	16	16	—	1	5.2 : 1	4.60	9.81	0.92	1.41	4.39
11/11/43	1211	56	25	16	—	3	5.1 : 1	4.18	9.63	0.88	1.13	4.28
19/12/43	2571	42	27	22	1	8	3.0 : 1	3.17	7.99	0.91	0.86	4.13
25/2/44	1405	63	16	19	2	T	3.8 : 1	3.21	7.48	1.33	0.65	2.73
27/3/44	732	52	11	21	6	T	1.7 : 1	4.33	8.52	1.18	0.87	3.50
19/4/44	996	69	7	24	T	T	3.2 : 1	4.76	9.03	1.05	1.02	3.99
30/5/44	785	70	11	19	—	T	4.3 : 1	4.69	9.07	0.74	1.04	3.94
B. No Return												
9/8/41	99	50	8	38	4	T	1.4 : 1	3.16	8.02	1.27	0.57	3.23
18/9/41	No Grazing	—	—	—	—	—	—	—	—	—	—	—
18/10/41	1712	32	6	60	2	T	0.5 : 1	4.11	10.12	1.74	1.32	3.92
3/12/41	2184	34	9	50	7	T	0.8 : 1	4.56	9.18	1.47	1.12	3.93
19/12/41	1132	22	7	54	17	—	0.4 : 1	4.86	9.14	1.49	1.09	3.61
26/1/42	1576	23	11	56	10	T	0.5 : 1	4.64	10.34	1.39	1.15	4.39
17/2/42	1173	50	8	38	4	T	1.4 : 1	4.57	9.17	1.47	1.08	3.62
16/3/42	1155	58	4	34	3	1	1.7 : 1	4.48	8.59	1.41	0.97	3.59
16/4/42	883	47	9	42	2	T	1.3 : 1	4.27	9.51	1.23	1.01	3.87
28/5/42	643	50	11	36	1	2	1.7 : 1	3.46	8.93	1.18	0.85	3.75

TABLE III. YIELDS OF HERBAGE FROM THE TREATMENTS, AND DETAILS OF CHEMICAL AND BOTANICAL ANALYSES AT EACH SAMPLE BULKING DATE—(Continued)

Date.	Dry Matter per Acre in Pounds.	Botanical Composition (per cent. Dry Matter).					Ratio of Grasses to Clovers	Chemical Analyses (per cent. Dry Matter).				
		Perennial Ryegrass.	Other Grasses.	White Clover.	Red Clover.	Other Species		Total N	Soluble Ash.	CaO	P_2O_5.	K_2O.
30/7/42	991	60	15	25	T	—	3.0 : 1	4.31	8.70	1.02	1.23	3.72
10/9/42	902	42	15	41	T	2	1.4 : 1	4.05	8.22	1.42	1.07	2.91
23/10/42	1835	50	10	37	1	2	1.6 : 1	4.72	8.40	1.40	1.12	2.86
15/11/42	730	41	19	34	3	3	1.6 : 1	4.47	8.32	1.43	1.02	2.88
19/12/42	2136	34	14	44	6	2	1.0 : 1	4.99	8.62	1.73	1.05	2.99
26/2/43	2256	24	7	62	6	1	0.5 : 1	4.59	8.93	1.44	0.90	2.65
26/3/43	803	26	20	50	3	1	0.9 : 1	4.90	8.82	1.40	0.84	3.09
20/5/43	466	53	14	32	T	1	2.0 : 1	4.06	7.95	1.23	0.79	2.64
2/9/43	1257	55	28	17	T	T	4.9 : 1	4.02	8.45	1.04	0.88	2.91
5/10/43	1506	41	18	40	T	1	1.5 : 1	4.97	8.82	1.54	1.38	3.24
26/10/43	1197	42	17	41	T	T	1.4 : 1	4.59	8.35	1.23	1.06	3.11
11/11/43	955	34	20	42	1	3	1.0 : 1	4.32	8.18	1.14	1.14	3.04
19/12/43	2582	33	20	44	3	–	1.1 : 1	4.05	7.93	1.54	0.94	3.23
25/2/44	1157	29	11	32	28	T	0.7 : 1	3.36	7.60	1.70	0.67	2.57
27/3/44	691	48	12	29	11		1.5 : 1	4.28	7.77	1.34	0.76	3.06
19/4/44	742	48	17	34	1		1.9 : 1	4.83	8.02	1.13	0.86	3.21
30/5/44	426	57	15	25	T	3	2.9 : 1	4.42	7.94	0.78	0.77	2.83
C. Urine Return												
9/8/41	239	76	10	13	1	T	6.1 : 1	3.67	7.47	0.80	0.67	3.40
18/9/41	1272	72	4	21	T	3	3.6 : 1	4.37	8.44	1.08	0.89	3.79
18/10/41	1171	61	6	29	2	2	2.1 : 1	4.46	10.03	1.19	1.50	4.80
3/12/41	2028	55	9	32	4	T	1.8 : 1	4.54	9.42	1.21	1.06	4.23
19/12/41	1459	31	8	51	10		0.6 : 1	4.73	9.71	1.31	1.03	4.36
26/1/42	1606	39	6	46	9	T	0.8 : 1	4.33	10.18	1.29	0.94	4.56
17/2/42	1273	49	4	44	3	T	1.1 : 1	4.62	10.33	1.35	0.95	4.52
16/3/42	825	47	4	44	1	4	1.1 : 1	4.74	9.56	1.40	0.87	4.08
16/4/42	815	52	7	41	T	T	1.4 : 1	4.34	10.26	1.13	1.01	4.54
28/5/42	594	54	4	42	T	T	1.4 : 1	4.35	9.43	1.06	0.82	4.11
30/7/42	1354	66	17	17	T		4.9 : 1	4.33	8.97	1.02	1.18	3.70
10/9/42	1401	54	13	33		T	2.0 : 1	3.97	9.00	1.28	1.07	3.25
23/10/42	2703	60	10	29	1	T	2.5 : 1	4.79	9.46	1.19	1.01	3.95
15/11/42	923	69	16	13	T	2	6.6 : 1	4.65	9.78	1.34	0.96	3.87
19/12/42	2785	58	7	33	2	T	1.9 : 1	4.76	9.66	1.42	0.93	3.88
26/2/43	2616	44	8	46	1	1	1.1 : 1	4.59	9.17	1.17	0.99	3.79
26/3/43	1000	53	7	39	1	T	1.5 : 1	4.64	9.52	1.28	0.76	3.88
20/5/43	762	61	11	28	T	T	2.6 : 1	4.09	8.50	1.05	0.77	3.18

TABLE III. YIELDS OF HERBAGE FROM THE TREATMENTS, AND DETAILS OF CHEMICAL AND BOTANICAL ANALYSES AT EACH SAMPLE BULKING DATE—(Continued)

Date.	Dry Matter per Acre in Pounds.	Botanical Composition (per cent. Dry Matter)					Ratio of Grasses to Clovers	Chemical Analyses (per cent. Dry Matter).				
		Perennial Ryegrass.	Other Grasses.	White Clover	Red Clover	Other Species.		Total N	Soluble Ash.	CaO	P_2O_5	K_2O.
2/9/43	1314	62	17	21	—	—	3.8 : 1	4.15	8.60	0.97	0.99	3.31
5/10/43	1933	49	15	36	T	T	1.8 : 1	5.33	10.16	1.51	1.56	3.91
26/10/43	1449	60	13	26	1	T	2.7 : 1	4.80	9.70	1.11	1.18	4.27
11/11/43	1106	52	17	31	T	T	2.2 : 1	4.52	9.75	1.02	0.99	4.00
19/12/43	2450	38	37	21	2	2	3.3 : 1	3.60	8.57	1.07	0.73	4.29
25/2/44	1063	24	20	45	8	3	0.8 : 1	3.28	7.62	1.41	0.54	2.75
27/3/44	511	50	10	35	5	—	1.5 : 1	4.50	8.40	1.24	0.80	3.48
19/4/44	900	53	11	35	—	1	1.8 : 1	4.87	9.17	0.99	0.79	3.96
30/5/44	477	70	12	18	—	T	4.6 : 1	4.27	8.45	0.72	0.80	3.28
D. Dung Return												
9/8/41	326	52	8	36	4	T	1.5 : 1	3.66	7.79	1.21	0.96	3.01
18/9/41	1266	43	11	43	3	T	1.2 : 1	4.58	8.20	1.44	1.09	3.44
18/10/41	1047	36	19	40	5	T	1.2 : 1	4.50	9.92	1.43	1.51	3.94
3/12/41	2256	46	19	31	4	T	1.9 : 1	4.32	9.02	1.27	1.22	4.31
19/12/41	1484	28	13	49	10	T	0.7 : 1	4.76	9.26	1.47	1.14	3.73
26/1/42	1691	30	21	39	10	T	1.0 : 1	4.53	9.59	1.46	1.14	3.80
17/2/42	1153	45	11	42	2	T	1.3 : 1	4.47	9.12	1.43	1.09	3.73
16/3/42	1433	42	7	47	2	2	1.0 : 1	4.49	8.92	1.44	1.02	3.67
16/4/42	1259	49	12	38	T	1	1.6 : 1	3.99	9.28	1.14	1.03	3.52
28/5/42	577	62	11	25	T	2	2.9 : 1	4.42	9.27	1.21	1.03	3.86
30/7/42	1665	58	22	20	T	—	4.0 : 1	4.38	8.46	1.06	1.27	3.41
10/9/42	1411	54	19	24	1	2	2.9 : 1	3.99	8.33	1.22	1.11	3.00
23/10/42	2355	57	18	23	1	1	3.1 : 1	4.40	8.43	1.20	1.19	3.24
15/11/42	795	44	24	30	1	1	2.2 : 1	4.14	8.65	1.33	1.16	3.04
19/12/42	2472	33	22	40	2	3	1.3 : 1	4.91	8.56	1.55	1.06	3.09
26/2/43	2795	27	25	39	9	T	1.1 : 1	4.83	8.84	1.37	1.13	3.06
26/3/43	945	32	20	36	1	2	1.7 : 1	4.87	8.86	1.28	1.01	3.26
20/5/43	719	60	22	16	1	1	4.9 : 1	3.99	8.26	1.24	0.85	2.86
2/9/43	1749	46	44	10	—	T	9.0 : 1	4.38	8.44	0.95	1.04	3.37
5/10/43	1520	59	20	21	T		3.8 : 1	4.96	8.67	1.29	1.46	3.38
26/10/43	1373	40	22	37	T	1	1.7 : 1	4.44	8.63	1.05	1.26	3.43
11/11/43	1074	36	30	31	1	2	2.1 : 1	3.99	8.54	1.01	1.17	3.27
19/12/43	2386	35	29	31	3	2	1.9 : 1	3.59	7.86	1.26	0.95	3.56
25/2/44	1446	28	27	30	15	T	1.2 : 1	3.28	7.15	1.65	0.62	2.36
27/3/44	698	42	25	26	5	2	2.2 : 1	4.23	7.71	1.30	0.76	2.97
19/4/44	926	42	18	39	1	—	1.5 : 1	4.63	8.03	1.19	0.87	3.15
30/5/44	718	73	9	17	T	1	4.8 : 1	4.74	8.21	0.94	1.05	3.40

The seasonal rise and fall of the proportion of clovers to grasses is clearly seen. White clover production is low during the winter and rises to a maximum during the October-December periods It is to be noted however that red clover declined markedly in importance after the first year, and showed up again only in the dry autumn of 1944. The increase in " Other grasses " is most marked and is probably associated with the change in management already mentioned. This increase was due largely to cocksfoot, the growth of which was most pronounced in the D.R. paddocks where grazing was not so severe from the beginning of the trial. Its growth was also favoured by lack of competition from ryegrass during the early stages of the trial (1).

When the ratios of total grasses to total clovers are compared, the position is even more clearly defined, as this eliminates the small proportion of weeds and the confusion of the various grass and clover species themselves.

Considering the chemical compositions of the herbage, there are similar changes throughout the seasons depending, no doubt, largely on the proportions of grasses to clovers. Thus where the clover growth increases, there is a rise in the calcium figure. Potash is higher in both the F.R. and U.R. treatments throughout and is probably the result of the larger quantities of available potash returned in the urine. These treatments however, show grass dominance as compared with the N.R. and the D.R., and the average higher percentage of potash in grass is probably a contributing factor.

The seasonal variability in chemical composition of pastures is well demonstrated in Table III. Thus the nitrogen (N) ranges from 3.16 to 5.33 per cent., calcium (CaO) from 0.66 to 1.74 per cent., phosphorus (P_2O_5) from 0.54 to 1.56 per cent. and potassium (K_2O) from 2.36 to 4.86 per cent. It is obviously difficult to draw any simple conclusion from such a variable set of figures, governed as it is by both botanical composition and growth conditions through the year. However, it is of considerable interest to note the low percentages recorded in all treatments in the summer of 1944 (the period 19th December, 1943, to 25th February, 1944), for all constituents other than calcium.

Table IV sets out the annual totals for the four year period. Total yields of botanical and chemical constituents have been calculated from the individual cut yields and the analyses of the bulked samples for the periods shown in Table III. In Table V these total production figures have been reduced to percentages, whilst the total dry matter productions are shown as relative quantities when the mean annual yield of the N.R. treatment is equated to 100. Considering these two tables together it can be seen that the yield of the N.R. paddocks has remained fairly constant at between 10,100 and 11,000 lb. dry matter per acre. This in itself is a very high yield and clearly shows the growth capabilities of a soil under good climatic conditions when good clovers and grasses are used and adequately fed with phosphates and lime. It is rather interesting that the annual yield of phosphorus is very close to the amount of phosphorus in the added superphosphate, but as there are no comparative plots without phosphate or without clovers, the value of these two in producing the strong growth of pasture as a whole cannot be assessed. Trials are at present under way at Palmerston North and Lincoln to obtain figures on this aspect.

TABLE IV. TOTAL YIELDS OF DRY MATTER AND BOTANICAL AND CHEMICAL CONSTITUENTS FOR THE TREATMENTS OVER THE FOUR YEAR PERIOD

Treatment.	Period.	Total Dry Matter (lb. per Acre).	Total Species Yields per Acre (in pounds).					Total Nutrients per Acre (in pounds).				
			Perennial Ryegrass.	Other Grasses.	White Clover	Red Clover	Other Species	N.	Soluble Ash.	CaO.	P_2O_5	K_2O
Full Return												
	22/7/40-21/7/41	13675	8903	651	2636	1236	249	643.5	1247.1	151.7	145.1	556.6
	21/7/41-28/5/42	15620	9399	1027	4711	229	254	702.2	1537.2	184.5	171.9	701.6
	28/5/42-20/5/43	13971	8742	1642	3146	66	375	621.7	1340.9	153.5	143.2	538.7
	20/5/43-30/5/44	12812	7777	2350	2259	98	328	531.6	1121.9	121.5	135.2	502.8
	Total	56078	34821	5670	12752	1629	1206	2499.0	5247.1	611.2	595.4	2299.7
	Average per year	14019	8705	1417	3188	407	302	624.8	1311.8	152.8	148.9	574.9
No Return												
	22/7/40-21/7/41	11140	4241	659	3609	2063	568	437.0	998.3	160.6	117.6	406.3
	21/7/41-28/5/42	10557	3946	849	5091	647	24	492.9	996.4	154.0	116.4	408.5
	28/5/42-20/5/43	10119	3914	1288	4434	327	156	466.8	868.5	145.4	103.0	297.4
	20/5/43-30/5/44	10513	4253	1933	3774	496	57	449.2	856.7	142.4	102.6	321.7
	Total	42329	16354	4729	16908	3533	805	1845.9	3719.9	602.4	439.6	1433.9
	Average per year	10582	4089	1182	4227	883	201	461.5	929.9	150.6	109.9	358.5
Urine Return												
	22/7/40-21/7/41	12586	7602	729	2821	1049	385	588.0	1151.8	146.7	127.8	529.4
	21/7/41-28/5/42	11282	5762	705	4276	444	95	506.3	1089.0	138.1	114.5	487.7
	28/5/42-20/5/43	13544	7672	1388	4321	119	44	616.9	1262.5	167.2	132.5	508.3
	20/5/43-30/5/44	11203	5459	2214	3265	175	90	487.4	1014.5	129.1	109.8	428.5
	Total	48615	26495	5036	14683	1787	614	2198.6	4517.8	581.1	484.6	1953.9
	Average per year	12154	6624	1259	3671	447	153	549.6	1129.4	145.3	121.1	488.5
Dung Return												
	22/7/40-21/7/41	12342	4024	570	4157	3257	334	493.7	1118.1	172.6	134.6	462.1
	21/7/41-28/5/42	12492	5147	1783	4945	563	54	553.2	1039.4	170.8	142.5	473.8
	28/5/42-20/5/43	13157	5724	2923	3987	365	158	596.8	1127.4	171.3	147.4	412.9
	20/5/43-30/5/44	11899	5088	3189	3173	344	105	495.8	968.4	142.3	123.0	386.1
	Total	49890	19983	8465	16262	4529	651	2139.5	4253.3	657.0	547.5	1734.9
	Average per year	12472	4996	2116	4065	1132	163	534.9	1063.3	164.2	136.9	433.7

Where urine and dung have been returned, the increase in yield has been 15 per cent. and 18 per cent. respectively and where both were returned the increase was 33 per cent., a very interesting coupling effect, quite apart from the degrees of increase themselves. Urine stimulated the grass in the early stages of the trial but the grass to clover ratio subsequently declined. This may possibly have been due to the drain of phosphate in the discarded dung and to the fact that, although there was ample nitrogen being returned in the urine, there was insufficient phosphate applied as superphosphate to allow the grasses to take full advantage of the other nutrients. There is no definite evidence of this from the trial itself, but the decrease in the grass to clover ratio, together with the relatively low phosphorus figure for the herbage as a whole is suggestive.

The dung gave no immediate stimulation to the grass constituents but acted largely to give increased clover growth in the early stages. The grass to clover ratio later increased, presumably due to action of the less soluble nitrogen constituents, and also to transference in the soil of nitrogen compounds from the clover-nodules to the grass plants. It is seen that the ratio of grasses to clovers rose from 0.62 : 1 to 2.35 : 1 over the four year period. The effect of returning the phosphate in the dung is reflected in the generally higher level of phosphorus than in the N.R. treatment where the botanical composition is somewhat similar, the average extra uptake of phosphorus being 27 lb. per annum. There is only a small difference to be seen in the total calcium figures, and presumably the added lime gave adequate supplies of this to all treatments, irrespective of the quantities in the droppings returned. The potash totals and percentages appear definitely greater than in the N.R. treatment, but are probably associated as much with the rising grass percentage as with the amount of returned potash in the dung.

In the F.R. treatment there is an overall increase in the grass constituents, and while the proportion of clovers is much lower than in the N.R. treatment, the difference in absolute amounts is not as marked. The absolute uptake of nutrients is greatest in all the constituents other than calcium, of which the greatest amount was taken up in the D.R. treatment. In this latter, however, there was not only a much greater total amount of clover (with less grass competition due to the lack of urine nitrogen) but also a similar return of calcium in the dung.

The seasonal spreads of production are shown in Table VI. The percentage figures shown have been calculated directly from the data in Table III by assuming that daily growth rates within a period were the same. By this means some period yields were divided to fit them into the seasonal groupings. This assumption is no doubt incorrect for single analyses, but probably gives a reasonable figure for the total four year period.

It can be seen that there is a better seasonal spread with the higher yielding paddocks, although the figures are not as impressive as early observations and figures indicated. Possibly, however, this latter impression was confused by the absolute amounts of herbage available in the different seasons. There is also the strong possibility that the ryegrass element in the return treatments was somewhat inhibited in development by the more lax grazing management towards the end of the trial. This would affect the seasonal spread markedly because of the

TABLE V. RELATIVE DRY MATTER YIELDS AND BOTANICAL AND CHEMICAL COMPOSITIONS FOR THE TREATMENTS OVER THE FOUR YEAR PERIOD

Treatment.	Period.	Relative Dry Matter. Av. N.R. = 100.	Botanical Composition (per cent. Dry Matter).					Ratio of Grasses to Clovers.	Chemical Composition (per cent. Dry Matter).				
			Perennial Ryegrass.	Other Grasses.	White Clover.	Red Clover.	Other Species.		N.	Soluble Ash	CaO.	P_2O_5.	K_2O.
Full Return	22/7/40-21/7/41	129.4	65.2	4.7	19.3	9.1	1.7	2.46 : 1	4.70	9.12	1.11	1.06	4.08
	21/7/41-28/5/42	147.8	60.1	6.6	30.2	1.5	1.6	2.10 : 1	4.49	9.84	1.18	1.10	4.49
	28/5/42-20/5/43	132.1	62.6	11.7	22.5	0.5	2.7	3.23 : 1	4.44	9.60	1.10	1.02	3.85
	20/5/43-30/5/44	121.1	60.7	18.3	17.6	0.8	2.6	4.29 : 1	4.15	8.75	0.95	1.06	3.92
	Total Period	132.6	62.1	10.1	22.7	2.9	2.2	2.8 : 1	4.46	9.34	1.09	1.06	4.10
No Return	22/7/40-21/7/41	105.4	38.0	5.9	32.5	18.5	5.1	0.85 : 1	3.92	8.96	1.44	1.06	3.64
	21/7/41-28/5/42	99.8	37.4	8.1	48.2	6.1	0.2	0.84 : 1	4.67	9.44	1.46	1.10	3.87
	28/5/42-20/5/43	95.7	38.7	12.7	43.8	3.2	1.6	1.09 : 1	4.61	8.58	1.44	1.02	2.94
	20/5/43-30/5/44	99.4	40.5	18.4	35.9	4.7	0.5	1.45 : 1	4.27	8.15	1.35	0.97	3.06
	Total Period	100	38.6	11.2	39.9	8.4	1.9	1.0 : 1	4.36	8.79	1.42	1.04	3.39
Urine Return	22/7/40-21/7/41	119.0	60.4	5.8	22.4	8.3	3.1	2.16 : 1	4.67	9.15	1.16	1.02	4.20
	21/7/41-28/5/42	106.6	51.1	6.2	37.9	3.9	0.9	1.37 : 1	4.49	9.65	1.22	1.01	4.32
	28/5/42-20/5/43	128.1	56.6	10.2	31.9	0.9	0.4	2.04 : 1	4.55	9.32	1.23	0.98	3.75
	20/5/43-30/5/44	106.0	48.7	19.8	29.1	1.6	0.8	2.23 : 1	4.35	9.06	1.15	0.98	3.82
	Total Period	114.9	54.5	10.4	30.2	3.7	1.2	1.9 : 1	4.52	9.29	1.19	1.00	4.02
Dung Return	22/7/40-21/7/41	116.8	32.6	4.6	33.7	26.4	2.7	0.62 : 1	4.00	9.06	1.40	1.09	3.74
	21/7/41-28/5/42	118.1	41.2	14.3	39.6	4.5	0.4	1.28 : 1	4.43	8.32	1.37	1.14	3.79
	28/5/42-20/5/43	124.3	43.5	22.2	30.3	2.8	1.2	1.98 : 1	4.54	8.57	1.30	1.12	3.14
	20/5/43-30/5/44	112.6	42.8	26.8	26.7	2.9	0.8	2.35 : 1	4.17	8.14	1.20	1.03	3.24
	Total Period	117.8	40.0	17.0	32.6	9.1	1.3	1.4 : 1	4.29	8.53	1.31	1.10	3.48

lack of that species (ryegrass) which performs so relatively well in the late autumn and winter periods. It will be appreciated that the greater winter percentages and the better overall spreads of the higher producing paddocks is a factor of considerable practical importance.

The outstanding feature of the whole set of figures is, of course, the general excellence of the spread in all treatments. Important as is the better spread of production when animal droppings are returned, the high productivity of the N.R. treatment when topdressed adequately with lime and phosphate and grown under good climatic conditions cannot be overlooked. The part played by the pedigree white clover growing under conditions which are ideal for its full expression, will readily be appreciated. Trials are in progress to obtain measurements on this aspect and the effect on associated grass growth.

There are, on the other hand, certain stock feeding difficulties apparently associated with clover dominance (notably bloat), and thus the production record itself cannot be the only guide to the ideal pasture sward, which appears to have grass dominance as its main criterion.

TABLE VI. SEASONAL SPREAD OF TOTAL DRY MATTER PRODUCTION FOR THE TREATMENTS OVER THE FOUR YEAR PERIOD

(All yields as per cent. total annual production of Dry Matter)

Treatment.	Season	Spring (Aug.-Oct.)	Summer (Nov.-Jan.)	Autumn. (Feb.-April)	Winter (May-July)
Full Return					
	1940-41	24.2	32.0	34.4	9.4
	1941-42	28.8	35.8	20.6	14.8
	1942-43	30.3	30.0	28.7	11.0
	1943-44	30.8	35.8	19.8	13.6
	Weighted Av for 4 years	28.6	33.4	25.7	12 3
No Return					
	1940-41	31.1	38.6	31.5	2 7
	1941-42	21.9	32.2	32.3	13.7
	1942-43	29 7	39.1	20.5	10.7
	1943-44	30 4	41.4	19.0	9.2
	Weighted Av. for 4 years	28.3	37.8	25 0	8.9
Urine Return					
	1940-41	26.6	34.4	32.0	7.0
	1941-42	25.2	36.4	24.6	13 8
	1942-43	33.6	36.6	20.3	9.5
	1943-44	34.0	38.8	15 5	11.7
	Weighted Av. for 4 years	29.8	36.5	23.3	10.4
Dung Return					
	1940-41	28.1	36.3	29.6	6.0
	1941-42	22.5	34.5	28.6	14.4
	1942-43	33.0	33.0	21.1	13.0
	1943-44	30.0	39.1	18.3	12.6
	Weighted Av. for 4 years	28.2	35.6	24.6	11.6

In Tables VII and VIII are detailed the dung and urine collections and their analyses. Full collections were carried out on the N.R. treatment throughout, and also the urine and dung from the D.R. and N.R. treatments respectively. In addition, two pilot sheep were measured fully on all treatments for the 1941-42 season. The quantitative figures for the dry matter in the dung are related to the corresponding period dry matter yields of the herbage, but the ratios for longer terms should be taken to obtain a better figure for dry matter digestibilities. The quantitative figures for urine are much more erratic, and as would be expected, are more dependent on rainfall and surface moisture conditions.

TABLE VII. DETAILS OF DUNG AND URINE COLLECTIONS FROM THE TREATMENTS, FOR THE PERIODS SHOWN

Period.	Dung Collections.						Urine Collections.		
	Dry Matter (Pounds per Acre).	Chemical Comp. (per cent. Dry Matter).					Pounds per Acre.	Chemical Comp. (per cent. Dry Matter).	
		N.	Sol. Ash.	CaO.	P_2O_5.	K_2O.		N.	K_2O.
Full Return									
22/7/41-9/8/41	75	3.0	13.6	3.8	3.7	2.8	721	1.36	0.96
18/9/41	389	3.1	13.4	4.3	3.6	1.7	5202	0.85	1.05
18/10/41	437	3.3	14.5	4.2	4.7	2.7	2889	0.97	0.88
3/12/41	605	3.5	14.9	4.5	4.6	2.1	7940	0.73	1.04
19/12/41	311	3.5	14.3	4.6	4.3	1.8	4842	1.01	1.59
26/1/42	484	3.5	13.7	4.7	4.1	1.5	5142	0.87	1.20
17/2/42	391	3.5	14.1	5.0	3.8	1.2	5263	1.18	1.54
16/3/42	495	3.0	13.0	4.7	3.5	0.8	3481	0.99	1.35
16/4/42	341	3.4	15.8	3.6	3.7	2.2	3935	0.63	0.88
28/5/42	374	3.5	15.1	2.4	2.1	1.5	3284	0.86	0.76
No Return									
22/7/41-9/8/41	48	2.7	12.6	3.1	2.8	2.8	347	0.95	0.76
18/9/41	No Grazing	—	—	—	—	—	—	—	—
18/10/41	338	3.1	15.5	5.7	4.5	1.3	4932	0.57	0.66
3/12/41	445	3.4	14.9	5.4	4.2	1.5	4811	0.70	0.93
19/12/41	304	3.4	14.9	5.6	4.2	1.4	3067	1.01	1.51
26/1/42	343	3.3	15.4	5.8	4.2	1.3	3009	0.87	1.15
17/2/42	264	3.4	15.1	5.6	3.9	1.3	2216	1.20	1.77
16/3/42	295	3.0	13.4	4.9	3.1	1.0	2833	1.13	1.42
16/4/42	193	3.0	14.5	4.8	3.8	1.3	2405	0.74	0.87
28/5/42	166	2.5	11.3	3.0	2.5	1.2	1983	0.59	0.65
21/5/43-2/9/43	281	3.2	14.4	4.9	3.5	1.2	2511	0.99	0.83
5/10/43	338	2.9	16.4	5.3	5.4	1.2	3115	0.91	0.83
26/10/43	226	3.0	13.9	4.1	4.0	1.2	2509	0.75	0.85
11/11/43	146	3.0	14.2	4.5	4.8	1.1	1676	0.92	0.91
19/12/43	500	3.1	12.7	4.9	3.2	1.2	3482	1.11	1.24
25/2/44	447	2.6	12.0	5.1	1.6	0.8	1496	1.54	1.67
27/3/44	180	3.0	12.5	4.6	2.9	0.9	1436	1.04	1.02
19/4/44	130	2.9	12.7	3.9	3.0	0.9	1262	1.10	1.01
30/5/44	97	2.8	12.5	3.4	3.8	1.3	1580	0.77	0.80
Urine Return									
22/7/41-9/8/41	61	2.8	13.0	3.5	3.1	1.7	467	1.03	0.90
18/9/41	159	3.4	13.7	4.8	3.6	1.8	2927	0.77	1.24
18/10/41	294	3.2	15.8	5.2	5.2	1.9	3195	0.64	0.72
3/12/41	525	3.5	14.1	4.8	4.1	1.7	5540	0.70	1.11
19/12/41	316	3.4	14.3	5.1	4.0	1.6	3162	1.03	1.39
26/1/42	366	3.3	14.5	5.4	3.9	1.4	4392	0.91	1.52
17/2/42	278	3.2	14.0	5.1	3.6	1.4	3092	1.13	1.60
16/3/42	369	2.9	12.9	4.6	3.0	1.1	3560	0.99	1.37
16/4/42	189	3.0	14.3	4.4	3.7	1.6	1954	0.69	0.85
28/5/42	169	2.4	11.3	3.0	2.2	1.1	2595	0.61	0.69
21/5/43-2/9/43	269	3.1	14.5	4.4	3.6	1.7			
5/10/43	247	3.2	16.5	5.3	5.7	1.5			
26/10/43	274	3.1	14.2	4.1	4.0	1.6			
11/11/43	161	3.2	13.4	4.2	4.8	1.5	Not Collected or sampled.		
19/12/43	617	3.1	12.0	4.2	3.1	1.4			
25/2/44	568	2.5	11.4	4.3	1.2	1.6			
27/3/44	245	2.9	12.9	4.6	2.3	1.5			
19/4/44	153	2.9	12.7	3.9	2.7	1.2			
30/5/44	95	2.8	11.3	3.1	2.8	1.0			
Dung Return									
22/7/41-9/8/41	45	3.0	13.2	4.1	3.2	1.1	678	1.01	0.81
18/9/41	237	3.3	13.6	4.6	3.7	1.3	2392	0.82	1.29
18/10/41	232	3.2	16.5	5.3	5.2	2.1	2978	0.72	0.77
3/12/41	507	3.4	14.5	4.8	4.6	1.7	5722	0.70	1.05
19/12/41	274	3.4	14.6	5.1	4.5	1.4	2730	1.12	1.45
26/1/42	291	3.4	14.8	5.3	4.5	1.1	3406	1.02	1.33
17/2/42	323	3.4	14.4	5.4	3.9	1.0	2564	1.22	1.69
16/3/42	323	3.0	13.1	4.8	3.3	0.9	3074	1.03	1.35
16/4/42	200	3.3	15.3	4.6	4.2	1.5	2881	0.73	0.92
28/5/42	258	2.6	11.5	3.0	2.8	1.1	2182	0.72	0.64
21/5/43-2/9/43							2905	1.04	0.91
5/10/43							2760	1.02	0.94
26/10/43							3101	0.76	0.91
11/11/43			Not Collected or sampled.				1742	0.83	0.81
19/12/43							3050	1.15	1.30
25/2/44							1523	1.47	1.49
27/3/44							1538	1.14	1.02
19/4/44							1602	1.19	1.08
30/5/44							1706	0.93	0.86

TABLE VIII. TOTAL AMOUNTS OF NUTRIENTS IN DUNG, AND SOLUBLE ASH IN THE DUNG AND URINE ESTIMATED TO HAVE BEEN PRODUCED DURING THE PERIODS SHOWN

(All weights in pounds per acre)

Treatment.	Period.	Dung.						Urine.		
		Total D.M. per acre.	N	Sol. Ash.	CaO.	P_2O_5.	K_2O.	Total Urine in lb. per acre.	N.	K_2O
FULL RETURN (Sampling Sheep only)	22/7/41-28/5/42	3902	131.0	498.6	166.4	151.0	67.9	42789	384.0	496.0
	Per cent	—	3.36	12.76	4.26	3.87	1.74	—	0.90	1.16
NO RETURN (All collected)	22/7/41-28/5/42	2396	76.4	—	125.2	93.3	32.1	25603	210.4	274.0
	Per cent.	—	3.19	—	5.23	3.89	1.34	—	0.82	1.07
	21/5/43-30/5/44	2345	69.0	—	111.6	80.0	25.5	19067	190.1	191.3
	Per cent.	—	2.94	—	4.76	3.41	1.09	—	1.00	1.01
URINE RETURN (All Dung collected. Sample sheep only for Urine measurements 1st period)	22/7/41-28/5/42	2726	87.1	—	130.7	102.9	41.5	30884	301.8	409.1
	Per cent.	—	3.20	—	4.80	3.78	1.52	—	0.98	1.32
	21/5/43-30/5/44	2629	77.3	—	113.4	81.0	39.3	—	—	—
	Per cent.	—	2.94	—	4.32	3.08	1.50	—	—	—
DUNG RETURN (All Urine collected. Sample sheep only for Dung measurements 1st period)	22/7/41-28/5/42	2690	88.8	—	128.8	109.7	36.0	28607	252.8	329.6
	Per cent.	—	3.30	—	4.79	4.08	1.34	—	0.89	1.15
	21/5/43-30/5/44	—	—	—	—	—	—	19927	206.3	204.6
	Per cent.	—	—	—	—	—	—	—	1.03	1.03

The chemical composition of the dung shows similar trends as does that of the pasture, although the total soluble ash figures are, of course, much higher. This is clearly seen in the phosphorus figure for the 1944 summer period, where the extremely low figure in the pasture is matched by an equally low phosphorus content in the dung.

As mentioned, the urine was not analysed for calcium and phosphorus due to its very low content. The figures for nitrogen and potassium show fairly wide differences, and this is probably a combined effect of the differences in composition of the ingested food and the dilution from surface and other water.

TABLE IX. BALANCES BETWEEN MEASUREMENTS OF HERBAGE CONSUMED, AND NUTRIENTS PASSED BY THE ANIMALS

Treatment.	Period.	Total Nutrients in Pounds per Acre.							
		N.		CaO.		P_2O_5.		K_2O.	
Full Return	21/7/41-28/5/42								
	In Herbage	702.2		184.5		171.9		701.6	
	,, Dung		131.0		166.4		151.0		67.9
	,, Urine		384.0		*		*		496.9
	Tota's	702.2	515.0	184.5	166.4	171.9	151.0	701.6	564.8
	Per cent. Balance	73.3		90.2		87.8		80.5	
No Return	21/12/40-21/7/41								
	In Herbage	242.8		89.3		57.7		207 4	
	,, Dung		67.8		89.8		70.1		28.2
	,, Urine		169.2		1.4		1.3		178.8
	Totals	242.8	237.0	89.3	91.2	57.7	71.4	207.4	207.0
	Per cent. Balance	97.6		102.1		123.6		99.8	
	21/7/41-28/5/42								
	In Herbage	492.9		154.0		116.4		408.5	
	,, Dung		76,4		125.2		93.3		32.1
	,, Urine		210.4		*		*		274.0
	Totals	492.9	286.8	154.0	125.2	116.4	93.3	408.5	306.1
	Per cent. Balance	58.2		81.3		80.2		74.9	
	20/5/43-30/5/44								
	In Herbage	449.2		142.4		102.6		321.7	
	,, Dung		69.0		111.6		80.0		25.5
	,, Urine		190.1		*		*		191.3
	Totals	449.2	259.1	142.4	111.6	102.6	80.0	321.7	216.8
	Per cent. Balance	57.7		78.4		78.0		67.4	
Urine Return	21/7/41-28/5/42								
	In Herbage	506.3		138.1		114.5		487.7	
	,, Dung		87.1		130.7		102.9		41.5
	,, Urine		301.8		*		*		409.1
	Totals	506.3	388.9	138.1	130.7	114.5	102.9	487.7	450.6
	Per cent. Balance	76.8		94.6		89.9		92.4	
	21/5/43-30/5/44								
	In Herbage	487.4		129.1		109.8		428.5	
	,, Dung		77.3		113.4		81.0		39.3
	,, Urine		†		†		†		†
	Per cent. Balance			87.9		73.8			
Dung Return	21/7/41-28/5/42								
	In Herbage	553.2		170.8		142.5		473.8	
	,, Dung		88.8		128.8		109.7		36.0
	,, Urine		252.8		*		*		329.6
	Totals	553.2	341.6	170.8	128.8	142.5	109.7	473.8	365.6
	Per cent. Balance	61.8		75.4		77.0		77.2	

* Not determined in analysis.

† No urine collections.

The results in Table IX represent an attempt to balance the measurements of ingested and excreted nutrients. Figures are presented for three years on the N.R., with one year on each of the others. The N.R. data are calculated from the complete collections of dung and urine on this treatment, whereas on the others, the results are obtained by the use of two sample sheep.

The balances illustrate some of the limitations of the experimental technique. The constituents which are excreted almost entirely in the dung, viz. calcium and phosphorus, show a reasonable degree of concordance (from 75-102 per cent. for calcium and from 74-124 per cent. for phosphorus).

Potash which is excreted to the extent of about 90 per cent. in the urine also gives balances in the same range (viz. 67-100 per cent.). The nitrogen balances are less satisfactory, the range being from 58-98 per cent.

There are several possible sources of error in the measurements of both ingestions and excretions by the sheep which could, cumulatively or separately, account for the differences found. These are :—

(a) The retention of some of the nutrients by the grazing animals for growth requirements could account for a small percentage, especially of the nitrogen. However, the sheep used were for the most part full-grown wethers and retention should not be great.

(b) There is a strong possibility of some retention as dung or urine by the sheep, due to the fact that it normally took much longer to harness up the animals than to unharness them. It is likely too, that the sheep were emptier when they went on to the block than when they came off, since the paddocks were not hard grazed at any time, while the sheep sometimes had come off fairly closely grazed areas.

(c) Quantitative urine collection is a difficult and laborious task, a point needing no elaboration to anyone who has attempted it. The fact that the balances for nutrients in the dung were better than those in the urine would suggest some loss of urine in the collection process itself.

(d) Urine samples held for analysis for periods of up to 2 months probably lost some nitrogen as ammonia. Herbivorous urine soon becomes distinctly alkaline in reaction and the loss of nitrogen as ammonia is suggested by the lower balance for nitrogen compared with potash.

(e) There is the possibility of a constant over-estimation of the ingested herbage by the cutting in the field. Although great care was always taken to do the cutting and hand plucking of the enclosed herbage down to the same stage as the grazing itself, this can be of course only an approximation. However, we feel that this point is not of such importance as the dung and urine losses, as normally such differences in cuttings tend to balance out over long periods.

Considered as a whole however, it is significant to note that with the exception of the phosphorus balance for N.R. in 1941, all the balances run the same way, and it is considered that the treatment differences can fairly be discussed in terms of pasture yields and compositions.

DISCUSSION

Most of the detail of the measurements in the trial have been discussed (1), and also comments have been made on each table supplied in the present paper. There remain some points on the overall results of the trial which merit repetition. Firstly, there is the very high and sustained yield on the N.R. treatment over the four year period. This has undoubtedly resulted from the exceptionally good growth of the pedigree white clover used and the fact that it was apparently adequately fed with the added phosphate and lime. Also because of the apparently high potash resources of the soil, non-return of this did not appear to cause any drop in production. Trials are at present under way at Palmerston North to obtain more detailed data of the effects of limiting certain of the minerals in such a soil as well as the influence of the clover itself.

Secondly there are the sustained increases in yield when the dung and urine are returned and the interesting combined total effect of these two together. The quick response by the grass to the return of urine is reflected both in the yield and composition. This effect, however, appears to have fallen off in intensity later in the trial, perhaps due to the drain in phosphate of the removed dung, which could have made less effective the nitrogen in the urine. However, there is no definite evidence of this from the trial results themselves.

The effect of the dung is clearly seen with its relatively slow build-up of the pasture sward from the stage of clover dominance to one of grass dominance, although even in the later stages there were still periods of poor grass growth. The return of the dung resulted in no loss of soil nutrients which are normally in short supply, viz., calcium and phosphorus, whilst an increase in soil nitrogen would follow both the strong growth of clovers and the more slowly available nitrogen of the dung itself. The loss of the nitrogen of the urine was reflected in the relatively slower growth of grass but the withholding of potash was probably not continued long enough to effect any limitation of pasture growth.

The trial does not give any clue as to whether or not the added phosphate and lime was necessary or beneficial, but, as the average annual extra uptake of phosphorus was only approximately 27 lb. it certainly does not appear to have been so.

Where both the dung and urine were returned, the total plane of production has been raised by 33 per cent., while the botanical composition is stabilized as a ryegrass dominant sward. The combined effect of the quick-acting urine and the slower but sustained dung effect is clearly seen. Again, there is no evidence of the real value of the added minerals, but it appears probable that for the grazing conditions of this treatment, much less added phosphate and lime could be used without loss of production. This, however, would need to be worked out under actual field trial conditions, using different classes of wet and dry stock with their varying mineral requirements for growth or production.

It is of interest to consider the nutrient turnover in the F.R. pasture and the manurial values determined for the mature dry stock used in the trial. For an average annual pasture yield of 14,000 lb. dry matter per acre, the quantities of the nutrients contained in the pasture sward expressed in terms of fertilizers are as follows:—

Nitrogen (N) equivalent to 27.9 cwt. sulphate of ammonia.
Calcium (CaO) equivalent to 2.4 cwt. carbonate of lime.
Potassium (K_2O) equivalent to 17.1 cwt. 30 per cent. potash salts.
Phosphorus (P_2O_5) equivalent to 6.5 cwt. superphosphate.

Of this amount, using dry sheep, and subject to the errors of the trial, we accounted for the following amounts actually contained in the dung and urine :—

Nitrogen (N) equivalent to 20.5 cwt. sulphate of ammonia.
Calcium (CaO) equivalent to 2.2 cwt. carbonate of lime.
Potassium (K_2O) equivalent to 13.8 cwt. 30 per cent. potash salts.
Phosphorus (P_2O_5) equivalent to 5.7 cwt. superphosphate.

As mentioned in the text these calculations are from the two pilot sheep on the F.R. treatment for the 1941 season only. It can be seen that much higher figures were obtained on some of the other blocks, but even so the amounts as shown do give a striking picture of the manurial value of, and the great need to utilize to the fullest advantage, the dung and urine produced by the grazing animal.

Looked at from the angle of the nutrient drain under conditions of no return of dung and urine, the quantities removed per acre per annum are as follows. For the average annual pasture yield of 10,600 lb. of dry matter, with a different botanical and chemical composition, the drain in terms of fertilizer is :—

Nitrogen (N) equivalent to 20.6 cwt. sulphate of ammonia.
Calcium (CaO) equivalent to 2.7 cwt. carbonate of lime.
Potassium (K_2O) equivalent to 11.9 cwt. 30 per cent. potash salts.
Phosphorus (P_2O_5) equivalent to 5.3 cwt. superphosphate.

The fact that the above trial was carried out on soil originally high in soil nutrients and under good climatic conditions undoubtedly accounts for the high yield of plant nutrients, and the practical interpretation of the results of the experiment must be made accordingly.

Thus it would be expected that the effects of dung and urine would be more marked on land which is not adequately supplied with the nutrients considered. This applies not only to the phosphate and lime which were applied liberally to all treatments of this trial, but also to the potash which apparently was satisfactory in the soil worked on, but which is passed in such large quantities through the soil-plant-animal cycle. Provided that other nutrient requirements are reasonably well catered for, it would appear from this trial that the nitrogen-fixing capacity of vigorous clovers is adequate to support good grass growth without nitrogenous topdressing, except for particular out-of-season needs. Trials are at present under way at Palmerston North to measure the effect considered in this trial, on pasture devoid of clovers, and on pastures of grass and clover not supplied with fertilizers as was this trial here reported.

For the overall practical implications of the effects of animal excreta on the pasture and the need not only for efficient conservation but also for a grazing management which will ensure the best redistribution on the farm, we refer to the foreword in the original paper.

There is little doubt that in New Zealand, both on flat land and on hill country, there is ample scope for improvements in grazing practices centring round the need to obtain the fullest redistribution of animal droppings. This will only come about when the farmer himself is made fully aware of the enormous fertility value of the excreta passed by the grazing animal. Linked with this would be carefully considered feeding out of silage, hay, and forage crops so that parts of the farm are not depleted of soil nutrients, without thought or care being given to the manurial return. Also, greater attention would be given to the fertilizer programme both on hill and flat country where, because of the grazing habits of the animals themselves, soil nutrients are transferred as dung and urine from one part of the paddock to another or from one part of the farm to another.

In all such cases, it follows quite logically that the application of fertilizer "from the bag" should be made to the depleted areas.

It is only by following such practices that balance is secured in the soil fertility level both of the paddocks as units, and the farm as a whole.

ACKNOWLEDGMENTS

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FERTILIZER BY FUSION OF ROCK PHOSPHATE WITH GREENSAND AND DOLOMITE

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Summary

Fusion, under reducing conditions at comparatively low temperatures, of rock phosphate with approximately its own weight both of roasted greensand and of limestone or dolomite, produces a soluble silico-phosphate slag comparable in composition with basic slag, and containing also a small proportion of available potash. This slag can also be used to cause "reversion" of superphosphate

INTRODUCTION

THE recent discovery by Macpherson (1) of a very large and accessible deposit of glauconitic rock in the North Island (at Otaihanga, near Paraparaumu) revives interest in the possible use of such greensand as a source of potash. Glauconite, of which high-grade greensand is almost entirely composed, is a silicate of iron and potassium containing approximately 20 per cent. of ferric oxide, 6-8 per cent. of potash, 50 per cent. of silica and 10 per cent. of combined water with minor amounts of alumina and magnesia. Roasting at 800°C. increases its potash content and prevents "sliming" in grinding, by expelling combined water.

Many attempts have been made to extract potash from greensand, varying from decomposition by sulphuric acid (2) to fusion with limestone and salt, while there have also been many attempts to make the phosphoric acid of rock phosphate more available to plants, ranging from the traditional use of acid to fusion with alkali or natural silicates such as olivine rock (3). However it seems from the literature available, that there has been no attempt to incorporate both the potash of glauconite and the phosphoric acid of rock phosphate into a soluble silico-phosphate slag by simply using the greensand as a flux for the rock.

EXPERIMENTAL

By preliminary small fusions of Nauru rock-phosphate, high-grade roasted greensand and marble with an oxy-acetylene torch on a graphite crucible-lid, it was found that, when the proportion of calcium carbonate was gradually increased to slightly more than one part by weight to one part greensand and one part rock, the mixture, while still readily fusible, gave a glassy green slag (containing occasional small globules of iron) which was now easy to grind, alkaline to phenolphthalein, and completely soluble in cold 2 per cent. citric acid solution. When the crushed slag was added to water through which was passed a slow stream of washed CO_2, approximately 16 per cent. of it, with a corresponding proportion of the phosphate, passed into solution overnight. This suggested the possibility of forming, under reducing conditions, at temperatures approximating those of a Portland cement kiln, a soluble slag comparable in P_2O_5 content with a good basic slag, and containing in addition about 2.5 per cent. of potash (K_2O).

It was found also that when dolomite replaced limestone in the mix rather less was needed and the mixture seemed more fusible.

Larger Fusions.

Larger quantities of mix (about 45 g.) were next heated, under more controllable conditions, in a fireclay crucible within a gas-fired ash-fusion furnace. Two experiments were then carried out :— (a) in which 1.1 parts of marble, (b) in which 1 part Collingwood dolomite, were heated with 1 part each of roasted Otaihanga greensand and rock phosphate, in a reducing atmosphere to 1,400 c. There resulted a very mobile slag (Fig. 1) cooling to a yellow-green translucent glass, which crushed to a very light green colour. In the standard A.O.A.C. test for citric acid solubility of phosphatic slags, in (a), 6.6 per cent. and in (b), 2.2 per cent. of the slag remained undissolved. These residues

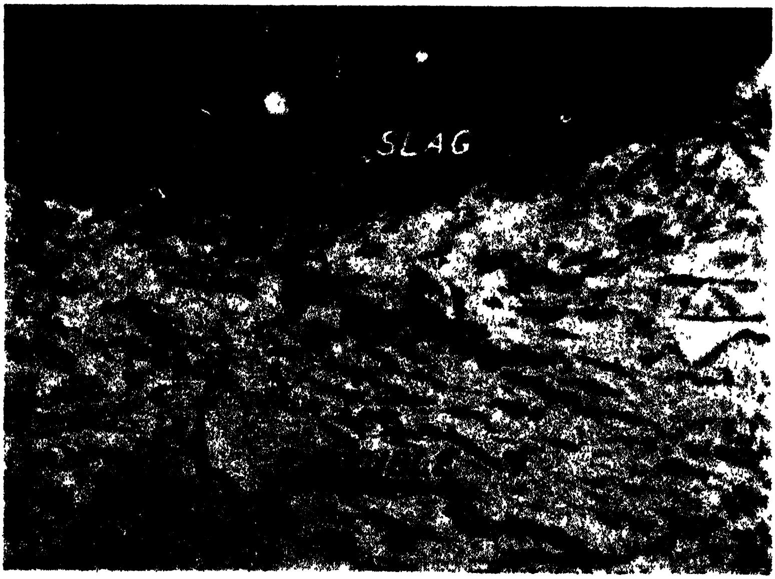

FIG. 1

contained approximately: (a), 15 per cent. and (b), 5 per cent. of the total P_2O_5, but no silica. The insoluble residues thus consisted, not of slag, but of the coarser particles of phosphate-rock, which had not been taken up and absorbed in the melt, and so later remained when the silicate slag itself dissolved in acid. On similarly heating another mix (containing 1 part dolomite) after first grinding its rock phosphate to pass a No. 100 British Standard Sieve, over 98 per cent. of the resulting slag passed into colourless solution while the residue contained much less than 2 per cent. of the total P_2O_5 (which amounted to 13.6 per cent. of the slag). Thus the first requisite for P_2O_5 solubility is that the rock phosphate be thoroughly dissolved in the slag, making the slag itself, and with it the contained phosphate, entirely citric-soluble. Possibly a second essential, were the slag made in larger bulk, would be sudden cooling to prevent recombination of fluor-apatite.

Oxidizing and Neutral Conditions of Fusion.

Since it was found that the total P_2O_5 content of the slags formed under reducing conditions as above was approximately only 85 per cent. of the amount expected, an attempt was made to reduce this loss of phosphorus through volatilization by heating the mixture (1 part dolomite) to 1,400°c. in an oxidizing atmosphere. The hot melt, which was now much less mobile, cooled to a duller and less translucent glass, somewhat vesicular, and of brown colour on crushing. In this slag, total P_2O_5 amounted to 14.6 per cent.- about 95 per cent. of the calculated value—but in the citric acid test only 72 per cent. of the slag, passed into yellowish solution, and the 28 per cent. residue was highly phosphatic. Even when the same mixture was heated in a neutral atmosphere to 1,400°c., the dull glass produced, which was slightly magnetic, and blue-grey in colour when ground, was almost equally disappointing in solubility. It appears that the greater fluidity and therefore greater dissolving power of the hot ferrous silicate slag produced under reducing conditions is essential for the absorption of the phosphate and for the citric solubility of both slag and contained phosphate. That only the ferrous slag is citric soluble is also shown by heating some of the crushed green glass in air, when it turns red by oxidation, and becomes quite insoluble. It is perhaps significant that in basic slag itself there is actually more ferrous oxide than here, as shown in the following comparison of the composition of an average basic slag (4) with that of the slag formed by fusing equal parts of rock phosphate, roasted greensand and dolomite in a reducing atmosphere : -

	Basic Slag	Silico-Phosphate Slag*
CaO per cent.	42	31.1
MgO ,, ...	6	8.3
Al_2O_3 ,, ...	2.5	3.0
Fe_2O_3 ,, ...	8.5	0.9
FeO ,, ...	13.5	8.3
SiO_2 ,, ...	8	30.2
P_2O_5 ,, ...	15	13.5
K_2O ,, ...	Nil	2.3

* Made by fusing together, without precaution against loss of phosphorus through reduction and volatilization, equal parts Nauru phosphate, roasted Otaihanga greensand and Collingwood dolomite.

Low Melting Point under Reducing Conditions.

As the preliminary experiments described above indicated that reducing conditions are essential, further investigations were confined to these. The melting point in a reducing atmosphere for a mix containing 1 part dolomite as determined on a cone of the material by optical pyrometer and Seger cones, was under 1,250°c. This suggested that perhaps 1,400°c. was an unnecessarily high temperature for fusion, though still low compared with the 1,450°-1,550°c. required for fusions with olivine rock (3). When, however, such a melt was held at 1,300°c. in reducing atmosphere for half an hour, it was found not to be fluid, the resulting glass was only 88 per cent. soluble, while the 12 per cent. residue contained at least 25 per cent. of the total P_2O_5. It thus seems that a temperature of 1,350°-1,450°c. is actually needed, not perhaps directly to break up the insoluble fluor-apatite molecule, but rather to make the slag sufficiently mobile to attack the phosphate and thus dissociate it. It is likely that a small proportion of boric acid in the mix (added as borax or as boron-containing coal-ash) could be used to lower this temperature considerably, so long as it did not make the molten slag too aggressive to furnace linings, or the resulting glass insoluble.

POTASH CONTENT

The K_2O content of the soluble slag was found to be 2.25 per cent. while 1.4 per cent. could be extracted with cold 5 per cent. acetic acid.

REVERSION

The alkaline soluble slag causes complete reversion, when mixed, warm and moist, with an equal weight of superphosphate. The resulting compound contains 17-18 per cent. P_2O_5 and slightly over 1 per cent. K_2O with 15-16.5 per cent. P_2O_5 citric soluble, and is thus superior to the serpentine superphosphate (5) previously suggested by the author, in that it contains slightly more P_2O_5 and an appreciable amount of potash.

POT TESTS

Outdoor pot trials were carried out with the slag using blue lupins in Maunu (N. Auckland) basalt soil (kindly supplied by Soil Bureau, D.S.I.R.) which is somewhat phosphate-fixing, although fairly fertile. The trials were done in triplicate while the control pots contained, like the others, 0.5 g. ammonium nitrate plus 10 g. calcium carbonate. Although, as indicated in Fig. 2, phosphate-response was not high, it showed up at least as well for slag as for superphosphate or serpentine superphosphate. (A remarkable feature of these trials on Maunu soil, in spite of liming, was the uptake of manganese in all four cases - namely 0.4-0.5 per cent. Mn on the ash, or 400-500 parts per million on dry weight.)

CONCLUSIONS

1. It seems that reducing conditions are required for producing, at comparatively low temperatures, slags of high citric solubility. Such conditions involve increased danger of loss of phosphorus, but in the Tennessee Valley Authority fusions of rock phosphate and olivine rock, where the carbon of the arc and furnace linings gave reduction at 1,450°-1,550°c., this loss was prevented by continuous working of the furnace, so that a cover of unfused charge above the molten material acted as an absorbent trap for vapours of P or P_2O_5 (3). The danger of loss is also lessened in our case by the lower fusion temperatures permitted.

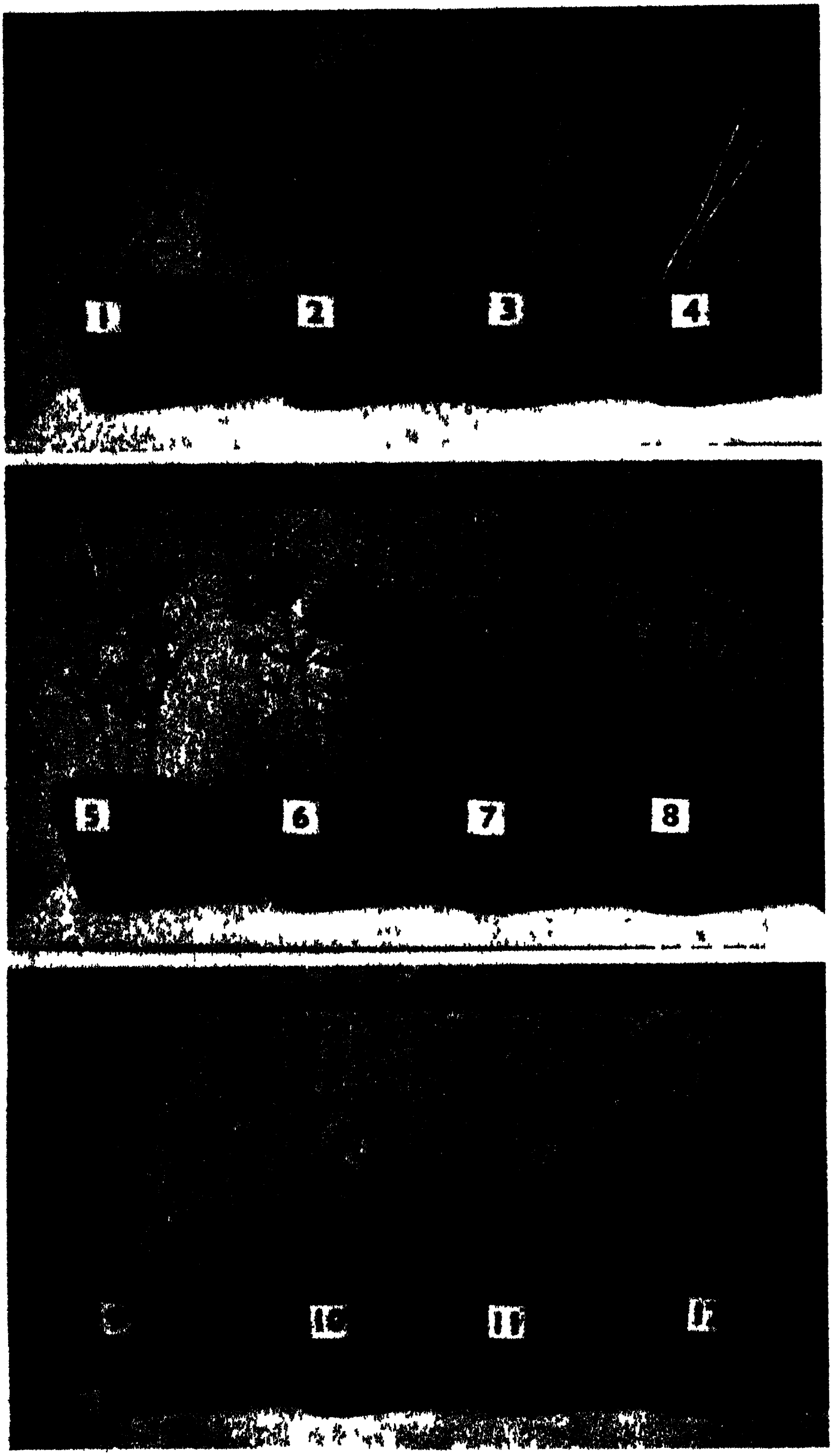

FIG 2

Control (C)	C + Super	C + Serp Super	C + Slag.
Dry wt 4.16 g	5.47 g	5.27 g	5.68 g

2. The incorporation of a small percentage of potash in the slag not only increases its value as a fertilizer but is also helpful as a flux ; indeed perhaps the main advantage gained by including greensand in the fusion lies in the possibility of producing at moderate temperatures and from plentiful native materials, a citric-soluble slag not unlike the best basic slag. This is the form of fertilizer in which phosphate is likely to be most effectively and economically expended, especially for pasture on acid, magnesia deficient, or phosphate-fixing soils--a claim which can be tested by producing the slag in quantity sufficient for pot and field trials.

EVIDENCE OF MOBILITY OF THE SLAG DURING FUSION

Indication of the highly fluid nature of the slag at 1,400 c. is its invasion of the spun fireclay-and-grog material of the assay-crucible, as illustrated in Fig. 1 where crucible and contents have been cooled and then broken across.

POT TEST, USING BLUE LUPINS

Growth outdoors in Wellington in 3 months August-October, 1947, with 0.75 g. of each fertilizer per pot of 500 g. soil.

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REVIEW

SVALOF 1886-1946; HISTORY AND PRESENT PROBLEMS

Edited by A. Akerman, Ph.D., O. Tedin, Ph.D., K. Froier, Ph.D. English Technical Editor, Dr. R. O. Whyte. Lund, 1948. 389pp. 30s

"The purpose of the present book is to present the activities of the Swedish Seed Association in the hope that such a presentation may intensify the international connexions of the association." In these words Lindberg, after outlining the work of the Cereal and Chemistry Laboratories, returns to the theme of the preface in which the editors stress the value of personal contacts between plant breeders from different parts of the world.

As the Swedish Seed Association was founded in produce crops and pastures especially adapted to Swedish conditions, the papers outlining the practical achievements in individual crops are of limited interest to us in New Zealand. Of considerable interest, however, are the general papers which outline the history and, to some extent, the theory of the breeding methods employed at Svalof:

The Breeding of Self-fertilized Plants by Crossing, by A. Akerman and J. MacKey.

Natural Selection and the Breeding of Cross-fertilized Plants, by G. Nilsson-Leissner.

The Cyto-Genetic Department, by A Levan.

Experiences from Work with Induced Polyploidy in Cereals, by A. Muntzing.

Mutation Work at Svalof, by A. Gustafsson and J. MacKey.

These authors are all well known for their papers in many scientific journals, and those accustomed to follow their work may be disappointed by the brevity of the theoretical discussions. For example, Akerman and MacKey's condensed account of the effect of Rasmusson's interaction hypothesis on the interpretation of frequency curves of yield data would only confuse readers who were not familiar with the theories discussed, while failure to mention developments of the interaction hypothesis later than those of Rasmusson, raises doubts in the minds of others. However, the accounts given show the way in which methods now used at Svalof have been developed and how the practical breeders of Svalof make use of recent advances in cytology and genetics. How they also contribute to advances of fundamental theory is not discussed, but the bibliographies, especially that at the end of Levan's article on the work of the Cyto-Genetic Department, list theoretical and practical papers by members of the staff.

As a means of strengthening international contacts, the final paper, discussing the availability of publications from Svalof, is useful.

The English translation has been made with great care and the book is excellently printed and produced.

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Editor: N. A. Marris, M.Sc., B.Com. (Assistant Editor: M. O'Connor, M.Sc.) Department of Scientific and Industrial Research, Wellington

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STUDIES IN MONOZYGOTIC CATTLE TWINS:

I. ORGANIZATION OF TWIN COLLECTION

By JOHN HANCOCK, Ruakura Animal Research Station,
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(*Received for publication, 8 April, 1949*)

Summary

(1) The organization of twin collection work at the Ruakura Animal Research Station is described.

(2) In five years, 1944-8 a total of 222 sets of monozygotic twins have been located. This annual number of 40-50 sets is adequate to justify large scale use of monozygotic twins for experimental studies.

(3) Methods of calculation of frequency of monozygotic twinning are described with reference to data of Johansson and Bonnier. Frequency rates are shown to be of major importance in twin collection work.

(4) The relation of distance, sex, collection methods, and year to the ratio of monozygotic twins in the inspected sample of cattle twins is discussed, in view of the usefulness of such data to workers attempting twin collection.

(5) Three factors on the organization side are essential to successful twin collection: (i) A relatively concentrated cattle population; (ii) Full time responsibility of a specialized worker; (iii) Enthusiastic farmer co-operation.

> "*A fundamental characteristic of living organisms is that they are alike in general plan, and different in detail. There are two corresponding schools of biologists, one of which emphasizes the average, and the other, the individual and particular. The rapid development and diffusions of statistical techniques in the biologic as well as in the physical sciences, tends to emphasize the average and the general; on the other hand, great discoveries and concepts of biology have resulted from scrutiny of the individual, of the detail. . . . . .*"
>
> S. BRODY *

INTRODUCTION

THE difficulties of controlled experimental work with cattle, spring mainly from two sources: (1) The inherited variability in most economically important characteristics, and (2) their great size. For practical reasons their size prevents the inclusion of a sufficient number of individuals to level out the uneveness of the experimental groups due to the great natural variability of the animals concerned. Thus sampling errors tend to be excessively high. The advent of modern statistical methods strongly emphasises this dilemma. In consequence, the general tendency of recent years has been for a research worker on cattle to concentrate on large scale field investigations rather than on controlled experimental work. However useful as indications to a problem the results of such survey work may be, they are often merely descriptive of a general situation and rarely gauge the effect of a specific factor, or a combination of such factors on a cattle population. Only the direct experimental approach can yield a precise analysis of particular causes and their effects.

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* Bioenergetics and Growth (1945)

Some of the success of plant research work can be attributed to the fortuitous situation that pure lines and clones have been available as experimental material. Such pure lines occur naturally in the form of self-fertilized plants. Some plants which normally are cross fertilized can be artificially self-pollinated. By this method, pure lines which do not occur spontaneously can be obtained rapidly. A comparable situation yielding homozygotic experimental material as clones is obtained by grafting, budding and by other methods of vegetative reproduction. Although pure lines of the higher animals can be produced, the difficulties are unfortunately greater than with plants. This is mainly due to three factors:

(1) Self-fertilization is not possible.

(2) Intervals between generations are long.

(3) Cost of keeping animals compared with plants is great.

That pure lines of mammals can be produced, has been shown by the success obtained with the Wistar Institute Rat, by King with mice and Wright with guinea pigs. Some progress has also been made with pigs at Cambridge and at U.S.A. experimental stations However, in spite of the evidence that intensive inbreeding is not necessarily detrimental to the vigour of animals, it is not likely that 100 per cent. inbred strains of dairy cattle will ever be approached. This becomes clear when it is considered that it would take ten generations or at least forty years, to obtain a line of cattle with an average inbreeding of approximately 87 per cent. (1). Many such lines may have to be started to find the one capable of development. In any event, animals of only one such line would be of limited usefulness for work on economically important problems, since, through such a line, the response of various environmental factors can be measured on only one specific genotype. It appears to be an almost impossible task to develop several such lines, each one representing a certain level of inherited productive capacity.

In view of these difficulties it is the more fortunate that artificial production of pure lines of dairy cattle need never be undertaken as their equivalents occur naturally as monozygotic twins.

The occurrence of monozygotic twins in man has been known for a long time and their potential usefulness as material for studies of genetical problems has been widely recognized (2). Although five sets of presumably monozygotic cattle twins had previously been described in the literature, it was not until 1932 that Kronacher (3), in an exhaustive study of 35 sets of like-sexed twins, finally demonstrated that monzygotic twins do occur in this species. Kronacher (4), summarizing his work, described 11 sets of monozygotic twins. Haak (5), described another eight sets, Chapman *et al.* (6), described one set and Lush (7), and Deakin (8), one each. Bonnier (9) (10), began research on cattle twins in Sweden in 1937 and in the four years 1941-4 he acquired for the Institute of Animal Breeding at Wiad, 34 sets.

Impressed by the possibilities arising from Bonnier's pioneer work, and following a visit to Sweden in 1938, McMeekan (11), initiated a search for monozygotic twins in New Zealand in 1941. On behalf of the Ruakura Animal Research Station, Dry of Massey College assumed responsibility for the North Island, and McMeekan for the South Island. Ruakura was able to receive one set of twins and one set of triplets in 1942, and four sets in 1943, mainly as a result of Dry's efforts. They were acquired when a few days old. Altogether Dry located 12 older sets up to 1944. In the South Island, thirteen sets of like-sexed twin calves were examined, none proving monozygotic. One set of four-year-old Shorthorn cows was located. Work was abandoned in the South Island because of the few dairy cattle available and the wide dispersal of the cattle population necessitating excessive travel under war-time conditions of transport. In 1944 the Ruakura Animal Research Station decided to begin a drive to ascertain whether a sufficient number of monozygotic twins could be acquired to make long term research, using such animals as material, a worthwhile undertaking. Though results had been disappointing to date it was considered that special organization on the part of a full time worker might give more promising results. The author was made responsible for the project.

METHOD OF COLLECTION

The advantages and difficulties of collection of twins under New Zealand conditions can be appreciated only if some of the peculiarities of the New Zealand dairy industry are considered. Because the dairy farmers of the North Island, where over 80 per cent. of the cows are located, rely almost exclusively on grass and grassland products as fodder for their cattle, the calving season is restricted to a relatively short period in the early Spring. The shortness of the calving season has two advantages from the point of view of collection of monozygotic twins:

(1) Twin calves collected in any one year are more or less of the same age.

(2) The work of collection does not necessarily interfere with other activities of a research worker as would be the case if calving were distributed evenly throughout the year.

It is easy to understand that as the calving season is the busiest time of the year for a dairy farmer in New Zealand he cannot undertake any tasks which are not strictly necessary to the orderly running of his farm. He cannot be expected to keep twin calves for inspection longer than the four days for which the law requires him to hold all calves before he sends them to the freezing works. It should be noted that most twin heifers and all twin bulls from commercial herds are disposed of as " bobby calves " or veal, when 4-7 days old. Early season twin heifers may be kept for replacement purposes and the farmer generally insists on having these inspected as soon as possible, so as to know how many more heifer calves he must retain should the inspected animals prove monozygotic and be sold for experimental purposes. As the majority of farmers are not prepared to make arrangements to send twin calves by rail, owing to the extra trouble involved, these must be collected on the farm.

In view of these difficulties which were increased by war-time transport shortages, it was decided to make an attempt to inspect twin calves upon their arrival at the freezing works. The Ruakura Animal Research Station is situated near Hamilton in the midst of one of the most intensive dairying areas in the world. A circle, with Ruakura as centre, and with a radius of twenty miles, includes approximately 160,000 dairy cows in milk or approximately 10 per cent. of the whole dairy cow population of the Dominion. A circle of forty miles radius includes 320,000 cows (12) (see Fig. 1). Most of the surplus calves from this area are slaughtered at the Auckland Farmers' Co-operative Freezing Works at Horotiu, situated ten miles from Ruakura. The arrangement for collection and transport of the calves are made by farmers' co-operative organizations known as " Bobby Calf Pools ". At these works, 170,000 calves are slaughtered annually, and as dairy farmers in New Zealand keep approximately 20-25 per cent. of all calves for replacements, this represents a dairy cow population of at least 230,000 cows.

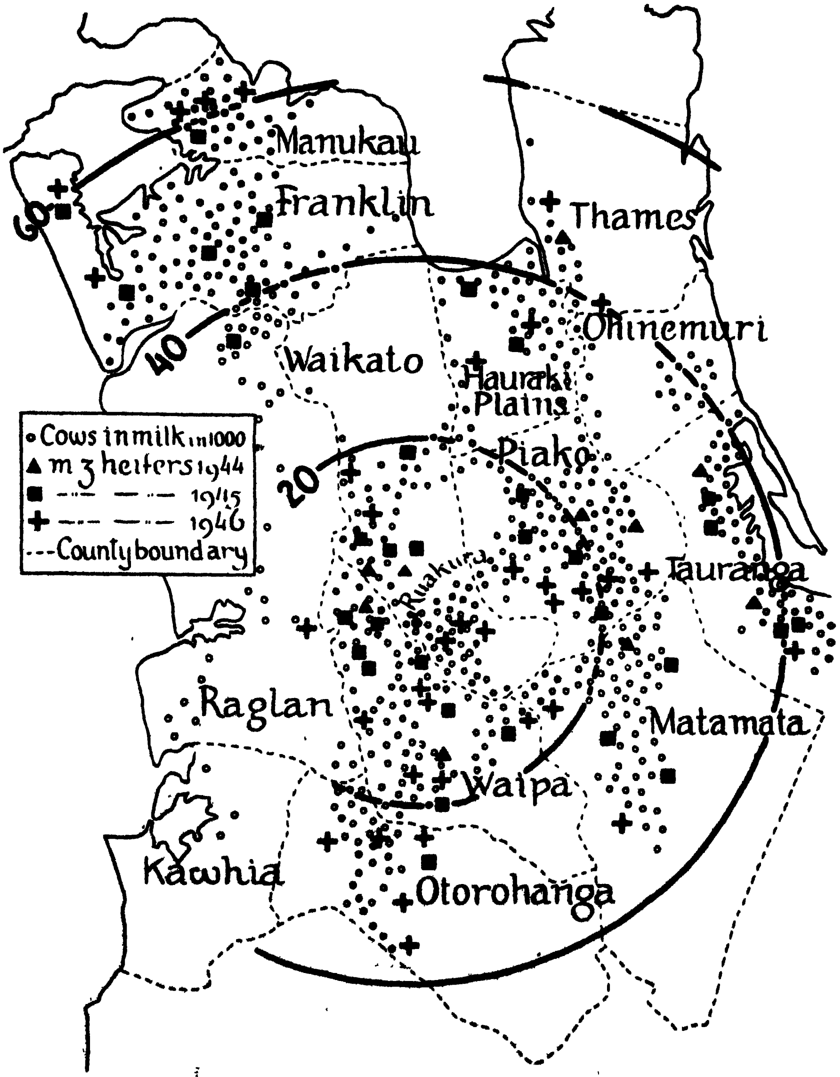

FIG. 1.—Distribution of the dairy cow population in the South Auckland and part of the North Auckland Land Districts, and the localities in which monozygotic twin heifers were found.

Through advertisements, and articles in the local press and agricultural journals, and per medium of the radio, farmers sending calves to Horotiu were asked to paint and label any like-sexed twins they thought were of monozygotic origin and to inform the drivers of the " Bobby Calf " lorries that the calves were twins which had to be sorted out on arrival at the Freezing Works for inspection by an officer from Ruakura. If the calves were to be transhipped by rail the lorry driver had to note on the railway consignment form that twins were included. This was necessary as it was not possible to arrange for a thorough search for eventual twins in every truck-load of calves that arrived at the Works. Farmers within a reasonable distance from Ruakura were asked to communicate directly with the Animal Research Station, and a promise of prompt inspection of the calves was made.

In the 1945 season the same method of collection was employed. In addition to the newspaper appeal, a leaflet containing advice on preliminary recognition of monozygotic twins was sent to practically all farmers supplying the Horotiu Freezing Works.

In view of the relatively few twins inspected at the Freezing Works in the previous year, (See Tables IVA and IVB) it was decided in 1946 to inspect all twins on the farm. A leaflet was again distributed. This method has proved very successful and has since been followed. It will be of interest to workers involved in twin collection to note the comparative failure of the approach through the Freezing Works. Initially, this appeared an ideal system but even in the first year when it was the only method advertised it accounted for only 50 per cent. of the twins examined. In the second year, the proportion fell to 20 per cent. During these two years it became increasingly clear that the factor of personal contact between research worker and farmer was of major importance. Farmers were more prepared to co-operate if and when they found the research worker was sufficiently interested to visit the farm and inspect the animals on the spot. Merely to despatch the animals to the works with labels attached was too impersonal, and appealed only to those interested in the greater financial return, should the calves prove monozygotic. On the other hand, personal inspection resulted in a situation where the farmer believed he was making a real and personal contribution to research.

NUMBERS COLLECTED

The following is a short summary showing the number of monozygotic twins of both sexes collected in these three seasons.

TABLE I. MONOZYGOTIC TWINS COLLECTED

	Heifers. (sets)	Bulls. (sets)	Total. (sets)
1944	15	9	24
1945	38	8	46
1946	37	5	42
1944/46	90	22	112

Prior to transferring five sets of heifer calves to the Dairy Research Institute, Palmerston North, in the Spring of 1946, the population of monozygotic twins at Ruakura reached 90 sets. Thus sufficient numbers of monozygotic twins can be located in New Zealand to make possible large scale projects, using these animals as experimental material. Collection work in 1947 and 1948 resulted in a further 99 sets of heifers, and eleven sets of bulls, making a total of two hundred and twenty-two sets located in the five years.

The number of monozygotic twins collected in the last three seasons was considered sufficient for the immediate needs. Based on an estimated requirement of 30 sets in milk, and 20 sets under special experimental conditions, the yearly replacements are calculated to be of the order of 25 to 30 sets, and it is considered that this number can be acquired easily without enlarging the present organization. If, however, a need for a larger number of monozygotic twins arises in the future, or if other research institutions decide to use such animals for experimental work, it is useful to know that there are three other areas in New Zealand apart from the Waikato, with a sufficiently large and concentrated cow population to be suitable sources of twin calves. These areas are: (1) North Auckland, with 354,000 cows; (2) Taranaki, with 215,000 cows; and (3) the Manawatu-Wellington area, with approximately 212,000 cows (12).

Finally, it may be pointed out that an intensification of the search for twins in the Waikato may result in a substantial increase in the number of monozygotic twins found annually. Fig. 1 has been prepared to show the density of the cow population in the counties within 60 miles of Ruakura. The location of the source of each set found during the search in the years 1944-6 has also been marked. If all counties had contributed the same number of twins relative to cow populations as the Waipa County a further 56 sets of heifer twins would have been acquired.

FREQUENCY OF MONOZYGOTIC TWINS

The frequency of monozygotic twins in a cattle population has a direct bearing upon the potential number available and upon the ease of collection. The number available is measurable by the ratio of monozygotic twins to total calvings, so that an assessment of this frequency is of primary importance. The ease of collection is related very closely in practice to the ratio of like-sexed twins to monozygotic, since the ratio determines the number of like-sexed twins that have to be examined for every monozygotic set located. Since the overall twinning rate in a cattle population directly affects the number of like-sexed twins born, it is rather important to determine whether there is a constant relationship between the overall twinning rate and the monozygotic twinning frequency. At first sight, a high overall twinning rate should make monozygotic twin collection easier, both in respect to numbers and differentiation. This will not be the case unless this increase is accompanied by more than a proportionate increase in the monozygotic twinning frequency, since without this, the number of twins that have to be examined for sets of monozygotic twins located is increased. Ideally, twin collection will be easy both in respect to number available, and ease of collection, if the monozygotic twinning frequency is high and the overall twinning rate is low.

Though the point has not been discussed by these authors, it is clear from the data of Johansson (13), and Bonnier (10) (14), that the overall twinning rate in cattle gives no indication of the rate of monozygotic twins in the same population. Working with three breeds in Sweden, both authors have collected data on the distribution of like-and unlike-sexed twins, while Johansson has reported the overall twinning frequency for these same breeds. By using the method employed by Strandkov *et al.* (15), of subtracting the like-sexed from the unlike-sexed twins, calculation of the number of monozygotic twins occurring in these samples has been attempted. This fifty-fifty method has given identical results in a human population to that which takes the sex ratio of twins into consideration (14). The results of applying the method to the Scandinavian material are shown in Table II.

TABLE II CALCULATED FREQUENCIES OF MONOZYGOTIC TWINS IN SWEDISH CATTLE BREEDS

Basis.	Swedish Fresian.		Swedish Red and White		Swedish Polled Landrace.	
	Johansson	Bonnier	Johansson	Bonnier.	Johansson	Bonnier
Whole population	0.054	0.084	0.128	0.10	0.243	0.290
All twins	1.62	2.50	6.94	5.34	13.43	16.00
Like-sexed twins	3.19	4.87	12 98	10.14	23.68	27.59
Overall-twinning per cent.	3.35	-	1.85	—	1.81	-

It will be noted that the Swedish Fresian breed, showing the highest overall twinning rate of 3.35 per cent. shows the lowest overall monozygotic twinning frequency of 0.54 to 0.84, while the Swedish Red and White and Swedish Polled Landrace with overall twinning rates of 1.85 and 1.81, show markedly different overall monozygotic frequencies. Both sets of data show comparable trends so that despite Bonnier's conclusion that the inter-bred differences in his own data were not statistically significant, it would appear that the ratio of the overall twinning rate to the monozygotic twinning frequency is by no means a constant. In arriving at his conclusion, Bonnier worked only on the ratio of monozygotic twins to all like-sexed twins and did not take into account Johansson's material. The point is of fundamental importance since it is related so closely to the difficulties of twin collection work. If Bonnier's conclusions are sound, the success of twin collection in New Zealand could be repeated in any cattle population. If, however, the view expressed above is valid, viz. that the rate of monozygotic twinning is not constant relative to the overall twinning rate, then the ease of twin collection will be quite variable, depending on the ratio of the two twinning rates in the cattle population under consideration. In this connection it is important to point out that the overall twinning frequency in New Zealand is very low, (0.60 to 1.0 per cent.) but presumably, the monozygotic twinning frequency is reasonably high. Data printed later suggests a rate of 0.1 per cent. for monozygotic heifer twins. The only New Zealand data available for twinning rates in cattle is that of Ward (16) (17). His analysis of a sample of approximately 27,000 calvings in 1945-6 gave the following results.

TABLE III. INCIDENCE AND SEX DISTRIBUTION OF TWINS

	Two Males	Male and Female.	Two Females.	Total.
Twin Births	63	117	82	267
Total Calvings				26,976
Twinning Rate per cent.				1.0

Calculation of the frequency of monozygotic twins from these data is rather dangerous in view of the markedly abnormal distribution of the three sex combinations, indicating an inexplicable deficiency of males. Ward (16), made such an estimate and reported a probable monozygotic twinning rate of 0.1 per cent. of all births. Unfortunately, application of the same method to Ward's data for 1946-7 (17), on a comparable sample gives a surplus of like-sexed twins of only one set in 25,400 calvings, a large proportion of which were located in the Ruakura territory. In this latter data a similar marked deficiency of males existed, while in the same season Ruakura collected 42 sets of monozygotic twins. It is therefore obvious that no reliable estimate of the monozygotic twinning frequency for New Zealand cattle can be obtained from Ward's material. Thus little weight can be given to Korkman's (18) use of these data to study twinning frequencies or to his conclusion that no breed differences exist in the monozygotic ratio. Strandkov's (15) analysis of monozygotic twinning frequencies in white and negro populations in the U.S.A. shows statistically significant racial differences. It is perhaps important to point out that differences of the type recorded here for cattle may have a regional, rather than a racial basis, the three breeds occupying markedly different regional areas in Sweden.

THE SIRE AS A POSSIBLE CAUSE OF MONOZYGOTIC TWINNING IN CATTLE

Although it is not intended to discuss generally the causes of monozygotic twinning in cattle, it seems worthwhile in this connection to put on record evidence that the sire, in some cases, may be involved. The evidence is based on four cases of bulls, each having sired two sets of monozygotic twins. In view of the low frequency of monozygotic twin births, these occurrences can hardly be ascribed to chance. Specific environmental factors obtaining on the farms concerned can also be ruled out since, considering the seasonal nature of calving in New Zealand, the twins were born at widely spaced time intervals.

The birth dates of the calves are set out below :-

Bull	Set No.	Birth Dates
1	I	17/7/45
	II	25/9/45
2	III	9/7/47
	IV	13/7/47
3	V	12/8/47
	VI	15/9/47
4	VII	30/9/47
	VIII	18/9/48

It can be seen that the two sets of twins sired by Bull 4 were born almost a whole year apart.

The conclusion seems inescapable that the sperms of a bull may be so constituted as to cause the fertilized ovum to split.

ANALYSIS OF THE RUAKURA SAMPLE

Data have been collected on the frequency of monozygotic twins amongst the like-sexed twins which have been made available for inspection by farmers in New Zealand. It must be emphasized at the outset that the like-sexed twins which have been inspected did not represent a random sample of all like-sexed twins born. Because farmers were asked to report only those like-sexed twins which were of similar appearance, especially as to colour and size, it is believed that the sample inspected contained a greater proportion of monozygotic twins than a truly random sample. Thus, the data are not suitable for calculation of the frequency of monozygotic twins in the twin population as a whole, but when analysed as to variations due to the sex of twins, year of collection, method of collection and location, they yield some interesting information about the ratios of like-sexed twins to monozygotic twins in an inspected sample. Such information may prove very useful to other workers in this field and is therefore presented here in some detail.

Tables IVA and IVB show the ratios of twins inspected to monozygotic twins for the three seasons concerned. The data for 1944 are not complete in that no information is available as to the distance from Ruakura travelled by the twins which were inspected at Horotiu. These data are also incomplete insofar as they cover only the first 96 sets of twins inspected that year.

DISTANCE FROM RUAKURA

The distance to Ruakura by road or rail from the farms where the twins were located strongly influenced the ratio of all twins to monozygotic twins in that the further from Ruakura sets of twins were located, the greater were the chances that they were monozygotic. The situation is graphically presented in Fig. 2.

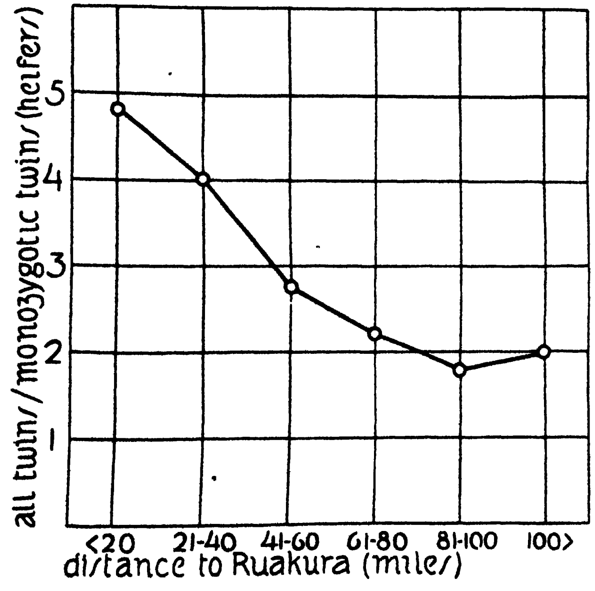

FIG. 2.—Relationship between distance of Ruakura from farms where twins were located, and the ratio of twins inspected to monozygotic twins.

The most likely explanation for this phenomenon is that farmers living further away from Ruakura tended to report a more selected sample of twins than farmers nearer to Ruakura. It is obvious that

TABLE IV. TWIN COLLECTION DATA

A. HEIFERS

Distance from Ruakura.	Inspected on Farms								Arrived by Rail						Inspected at Horotiu						Total 1944		Total 1945		Total 1946		Grand Total.	
	1944		1945		1946		Total		1944		1945		Total		1944		1945		Total.									
Miles.	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
20 or under	20	3	38	6	37	11	95	20	0	0	0	0	0	0	x	x	6	1	x	x	x	x	44	7	37	11	101	21
21-40	2	2	29	8	40	10	71	20	1	0	2	0	3	0	x	x	6	0	x	x	x	x	37	8	40	10	80	20
41-60	0	0	3	0	20	7	23	7	3	1	6	4	9	5	x	x	1	0	x	x	x	x	10	4	20	7	33	12
61-80	0	0	11	5	12	7	23	12	2	1	7	2	9	3	x	x	5	2	x	x	x	x	23	9	12	7	37	17
81-100	0	0	0	0	3	1	3	1	0	0	4	3	4	3	x	x	0	0	x	x	x	x	4	3	3	1	7	4
101 or more	0	0	7	4	1	0	8	4	4	2	6	3	10	5	x	x	0	0	x	x	x	x	13	7	1	0	18	9
Total	22	5	88	23	113	36	223	64	10	4	25	12	35	16	22	2	18	3	40	5	54	11	131	38	113	36	298	85
Ratio per cent.	4.4		3.8		3.1		3.5		2.5		2.1		2.2		11.0		6.0		8.0		4.9		3.4		3.1		3.5	

B. BULLS

Distance from Ruakura.	Inspected on Farms								Arrived by Rail						Inspected at Horotiu						Total 1944		Total 1945		Total 1946		Grand Total	
	1944		1945		1946		Total		1944		1945		Total		1944		1945		Total									
Miles.	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
20 or under	14	2	14	3	13	1	41	6	0	0	0	0	0	0	x	x	4	0	x	x	x	x	18	3	13	1	45	6
21-40	0	0	10	0	19	2	29	2	1	0	2	0	3	0	x	x	6	1	x	x	x	x	18	1	19	2	38	3
41-60	0	0	1	0	2	1	3	1	2	0	1	0	3	0	x	x	3	2	x	x	x	x	5	2	2	1	9	3
61-80	0	0	2	1	0	0	2	1	0	0	2	1	2	1	x	x	0	0	x	x	x	x	4	2	0	0	4	2
81-100	0	0	0	0	1	0	1	0	0	0	0	0	0	0	x	x	2	0	x	x	x	x	2	0	1	0	3	0
101 or more	0	0	0	0	1	1	1	1	1	0	0	0	1	0	x	x	0	0	x	x	x	x	0	0	1	1	2	1
Total	14	2	27	4	36	5	77	11	4	0	5	1	9	1	24	4	15	3	39	7	42	6	47	8	36	5	125	19
Ratio per cent.	7.0		6.8		7.2		7.0						9.0		6.0		5.0		5.6		7.0		5.9		7.2		6.6	

x Indicates data not available. a Shows number of sets inspected b Shows number of monozygotic sets inspected.

the increasing difficulties either to the farmer himself or to the inspecting officer, made more distant farmers examine the twins more carefully.

The sample of heifer twins located within 20 miles of Ruakura, which may be considered the least selected one, provides a basis for an estimate of the maximum number of monozygotic twins born in the Waikato dairy cow population. Assuming that one per cent. of all calvings result in twins and that approximately 25 per cent. of all twins are heifer-heifer twins, it can be calculated that with one set of monozygotic twins in every 4.8 sets inspected, there are no more than one set of monozygotic heifers born in approximately 2,000 calvings.

SEX

On analysing the data it soon became evident that bull calves had to be treated as a separate group, as they showed much lower ratio (1 : 6.6) than the heifer calves (1 : 3.5). There may be a number of possible reasons for this phenomenon of which the more likely ones are discussed below.

In view of the fact that, as shown above, the further from Ruakura sets of twins were located the greater were the chances that they were monozygotic, it was thought that if the bull twins were found to be located on farms on an average nearer to Ruakura than were the heifer twins, the differences in the respective ratios of twins inspected to monozygotic twins may not have been a real one. However, as 60.7 per cent. of the bulls compared with 66.4 per cent. of the heifers were inspected within 40 miles of Ruakura, this cannot have been a major factor causing the discrepancy in the ratios. That there was a real difference in the ratios for bull and heifer twins is also suggested by the fact that, of the heifer twins located on farms within 20 miles of Ruakura, which previously has been shown to be the least selected sample, one in every 4.8 was monozygotic compared with one in every 7.5 for the bulls.

Farmers may have tended to report bull twins on an average at a lower age than heifer twins, thus not having had the same opportunity to keep them under surveillence and, therefore, reported to Ruakura relatively more dizygotic bull twins than heifer twins. At first sight there seemed to be some grounds for this theory in that the average age (4.0 days) at which the bulls had been reported actually was slightly lower than that of the heifer twins (average 5.2 days). The distribution of the age classes in the two twin populations comprising 155 sets of heifers and 36 sets of bulls inspected in 1946 is shown in Fig. 3.* However, it has also been possible to calculate the proportion of monozygotic twins in the various age classes of heifers and as Fig. 3 shows, there is no apparent correlation between the age at which they were reported to Ruakura and the proportion of monozygotic sets. This does not however, exclude the possibility that some other factor of

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* A set of pedigree bull twins which had been reported at age of three and a half months is not included in the above data as it was far older than any other set, and its inclusion would have given a false impression of the average age of the bull twins.

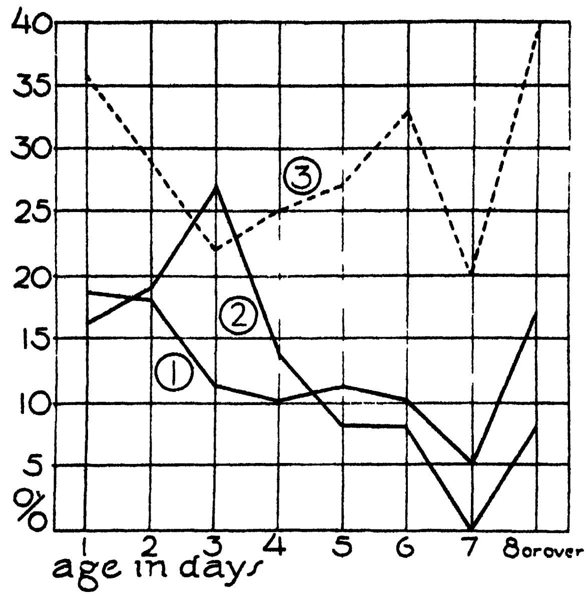

FIG. 3.—(1) Frequency distribution of the ages at which twin heifers were inspected.

(2) Frequency distribution of the ages at which twin bulls were inspected

(3) Percentage of monozygotic twin heifers calculated on all twin heifers inspected at various ages

a psychological nature so influenced the farmers that they tended to report to Ruakura a more unselected sample of bull twins than of heifer twins.

Greater pre-natal or early post-natal death rates of monozygotic bull twins are, of course, factors which could explain the discrepancy. The only evidence that would tend to support this theory is the fact that the secondary sex ratio in cattle is lower in twins than in singles (Johansson [13] and Ward [16] [17]), a fact however, which may be due to various other reasons than that there are relatively fewer monozygotic bulls in the twin population. It is believed that only a direct inspection of a large sized unselected sample of like-sexed twins will clarify this question.

Because of the relatively small number of bull twins which have been inspected, the discussion will hereafter be limited to the heifers.

METHOD OF COLLECTION

As previously mentioned, three different methods of collection were employed in 1944-5.

(1) Selection of twins on arrival at the freezing works;
(2) Selection of twins on the farms;
(3) Direct consignment of twins to the research station by the farmer.

The relative number of monozygotic twins was smallest in the first group, and greatest in the last, the ratios of all twins inspected to monozygotic twins being 8.0, 3.9 and 2.2 respectively. This situation could probably best be interpreted as due to the reluctance on the part of the farmers to send *via* the freezing works the twins they were convinced were monozygotic and instead, asked for inspection on the farms. It is not possible to decide whether the high proportion of monozygotic heifer twins in the sample of twins that arrived by rail was due to the method of collection, or the fact that most of these twins came from farms situated forty or more miles from Ruakura.

YEAR

It is important to have some idea as to whether availability of twins varies from year to year. There are two reasons why the 1944 sample cannot be compared with those of 1945 and 1946. Only the first 96 sets of twins are included in 1944. This may have had some bearing on the number of monozygotic twins found, as it is possible that later in the season the relative number of monozygotic sets may have increased or decreased. In addition, the twins were located on farms which were on an average nearer Ruakura than was the case in 1945 and 1946.

In the two comparable years there was slightly more monozygotic twins in 1946 than in 1945. One in every 3.1 sets of like-sexed heifer twins inspected in 1946 was considered monozygotic compared with one in every 3.5 in 1945. The difference is unlikely to be significant.

ESSENTIALS OF ORGANIZATION

The comparative failure of the first four years of twin collection work as contrasted with its marked success in the last four years has emphasized the importance of certain aspects of organization. From the experience gained it is fairly evident that at least three factors on the organization side are important if twins are to be obtained in adequate numbers to permit their effective use as material for cattle experiments.

(1) A relatively concentrated cattle population.
(2) Full time responsibility (seasonal) of a specialized worker.
(3) Enthusiastic farmer co-operation.

In the first four years none of these essentials was fulfilled. The cattle populations worked with were relatively widely dispersed. The workers concerned could direct but a small amount of time to the task, and in practice were available merely for recognition purposes. Farmer interest on a wide scale could not be obtained, partly because the first two essentials could not be met, and partly because the pressure of the early war years made it difficult to interest farmers in a project, the potential worth of which was difficult to grasp and which appeared to be of dubious value in the face of possible invasion of New Zealand by enemy forces.

During the past five years, concentration on a large compact cow population by one worker with full responsibility for all phases of the work during a period when farmers were emerging from war time pressure so that they could be approached successfully on farming matters, provided a much more satisfactory background for collection. These points seem worth stressing in this general account since the poor results in early years were almost responsible for abandonment of the project in New Zealand. It was only a firm belief in the very great potential value of monozygotic twins on the part of the controlling officers of the Animal Research Division, together with a realization of the practical difficulties involved, that resulted in the author being given the opportunity of full time organization of twin collection. These points should not be overlooked by institutions seeking this type of experimental material.

No reference has been made to the method of recognition of identical twins—obviously a factor of fundamental importance. This aspect is so wide in its scope and involves data so extensive that it will be published as Part II of this series of studies of monozygotic cattle twins.

ACKNOWLEDGMENTS

The successful outcome of work of the character described in this paper depends naturally on the assistance and co-operation of a great many persons. Special thanks are due to Dr C. P. McMeekan, Superintendent of the Ruakura Animal Research Station, without whose enthusiastic and unfailing support this work could not have been carried out. It is a pleasant duty to acknowledge the willing co-operation of the officers of the various Calf pools and Horotiu Freezing Works. Thanks are also due to Mr M. N. Wallace who assisted the author in the later stages of this work. Finally, thanks are due to all farmers who made their calves available for inspection.

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THE EFFECT OF ROOTSTOCKS AND INTERMEDIATE SCION VARIETIES ON THE COOL-STORAGE DISORDER, CORE-FLUSH, IN GRANNY SMITH APPLES

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Summary

Core-flush, a form of internal tissue collapse, is a major disorder of Granny Smith apples in cool store. Different root-stocks and intermediate scion varieties showed no consistent effect on the incidence of this disorder. There were wide seasonal variations in the incidence of core-flush, in successive crops from trees under test.

Granny Smith apples are particularly subject to core-flush in cool storage, this condition occurring earlier in the storage life of this fruit than any other disorder. In addition to normal Granny Smith plantings, many varieties have been reworked with this apple. Some of these varieties are themselves particularly prone to core-flush in cool storage, and it has been suggested that the inclusion of such intermediate scions might influence the amount of this disorder occurring in Granny Smith.

Description

Core-flush is a cool storage disorder which affects the flesh between seed cavities, causing a red-brown discoloration. At first this is slight, but in extreme cases affected tissue becomes discoloured and desiccated while cracking within the core zone sometimes occurs. This is accentuated by holding fruit below the recommended temperature and is most common in immature fruit. Smock (1946) has shown that considerable reduction has been obtained when susceptible apples were withheld from cool storage for periods up to 20 days. From data as yet unpublished it has been found that a reduction has been obtained when Granny Smith apples were withheld from cool storage for periods of five to six weeks, but this is not a practical means of control. The varieties, Dunn's Seedling, Granny Smith, and Statesman were particularly susceptible, while it is found to a lesser extent in Cox's Orange, Delicious, Jonathan, and Sturmer Pippin.

Experimental, 1944 and 1945

In 1944 and 1945 fruit from many combinations of rootstock—Granny Smith scion, and rootstock—intermediate scion variety—Granny Smith scion were collected and cool-stored. To obtain results under normal harvesting conditions, picking was left to individual growers to fit in with their own harvesting programme. With a few exceptions, all samples were picked within a few days of one another. Samples consisted of two bushel cases in 1944 and four bushel cases in 1945; all were stored at 34°F. and were removed from storage at approximately the same time. Every apple was cut in half and the degree of core-flush assessed. For record purposes four grades were used—"trace," "slight," "moderate," and "severe." The last two categories were classed as commercially significant. All samples were examined in 1944 between 24th and 28th of November, and in 1945 between 12th and 15th of November.

TABLE I HASTINGS DISTRICT PERCENTAGES OF TOTAL AND COMMERCIALLY SIGNIFICANT COREFLUSH ON REMOVAL FROM STORAGE

Orchard	Root Stock and Intermediate Scion Combination	1944						1945						
		Total Apples per Sample	Picking Date	Storage Date	Period of Storage (days)	Per cent Total Core flush	Per cent Commercially significant core flush	Orchard	Total Apples per Sample	Picking Date	Storage Date	Period of Storage (days)	Per cent Total Core flush	Per cent Commercially significant core-flush.
A	Northern Spy- Cox's Orange Pippin	263	24 4	27 4	213	90	57	A	499	22 4	30 4	197	70	44
B	Northern Spy- Dunn's Seedling	250	20 4	27 4	213	89	25	B	497	23 4	30 4	197	84	48
C	Northern Spy- Jonathan	251	22 4	27 4	213	97	52	C	500	17 4	20 4	208	88	62
D	Northern Spy- Delicious	250	20 4	27 4	213	94	60	I	497	16 4	23 4	210	84	58
E	Northern Spy- Statesman	250	20 4	27 4	214	94	74			—	—	—	—	—
F	Northern Spy- Sturmer Pippin	250	21 4	27 4	213	92	57	F	500	25 4	30 4	195	79	58
G	Northern Spy- Dougherty	250	22 4	27 4	213	88	58	J	496	30 4	2 5	195	77	32
H	Northern Spy Reinette du Canada	250	19 4	27 4	209	80	21	H	472	23 4	26 4	201	88	52
	Northern Spy	—	–					K	500	30 4	1 5	194	66	20

TABLE II. NELSON DISTRICT. PERCENTAGES OF TOTAL AND COMMERCIALLY SIGNIFICANT COREFLUSH ON REMOVAL FROM STORAGE.

		1944						1945						
Orchard.	Root Stock and Intermediate Scion Combination.	Total Apples per Sample	Picking Date	Storage Date	Period of Storage (days).	Per cent. Total Core-flush	Per cent. Commercially significant core-flush.	Orchard.	Total Apples per Sample	Picking Date.	Storage Date.	Period of Storage (days).	Per cent. Total Core-flush.	Per cent. Commercially significant core-flush.
1	Northern Spy	250	11.4	15.4	226	98	83	1	495	15.4	18.4	210	100	90
	Large's Seedling	—	—	—	—	—	—		452	15.4	18.4	212	82	60
2	Large's Seedling	250	10.4	15.4	226	91	53	2	474	15.4	18.4	210	95	65
3	East Malling XII	238	24.4	27.4	213	87	25	-	—	—	—	—	—	—
	East Malling XVI	188	24.4	27.4	209	86	22	-	-	—	—	—	—	—
4	Northern Spy- Cox's Orange Pippin	250	17.4	21.4	220	98	70	4	471	13.4	20.4	208	95	74
5	Northern Spy- Dunn's Seedling	250	12.4	27.4	213	98	69	5	495	8.4	14.4	212	99	94
6	Ivory's Double-Dunn's Vigour Seedling	-	-		-	-		6	494	13.4	16.4	212	97	66
7	Northern Spy- Jonathan	250	20.4	27.4	213	97	66	-	-			-	—	—
8	Northern Spy- Delicious	250	11.4	15.4	226	96	63	8	499	11.4	16.4	215	99	93
9	Northern Spy- Delicious	250	17.4	21.4	220	98	78		-	-		-	-	—
	Northern Spy- Sturmer Pippin	250	17.4	21.4	219	96	62		-			-	-	-
10	Northern Spy- King David	250	17.4	21.4	220	90	33	10	496	21.4	28.4	197	90	55
11	Northern Spy-King David-Washington	250	24.4	27.4	209	82	45	11	500	7.5	15.5	181	78	36
12	Northern Spy- London Pippin	250	11.4	15.4	226	85	41	12	499	10.4	16.4	212	98	83
13	Northern Spy- Statesman + Borax	226	20.4	27.4	209	93	50			-	-	-	-	
	Northern Spy- Statesman No Borax	226	20.4	27.4	213	85	26	12	502	10.4	16.4	214	91	66
14	Northern Spy- Dougherty	250	10.4	15.4	226	78	30	14	495	30.4	2.5	196	96	75
15	Northern Spy- Reinette du Canada	250	2.5	5.5	202	70	20	15	499	5.5	8.5	192	92	52

RESULTS, 1944 AND 1945

Percentages of core-flush in the Hastings fruit are given in Table I, those for the Nelson samples in Table II.

Incidence of core-flush was high in all samples in both years, though the amount varied from year to year in fruit from the same trees. For any particular combination of rootstock and intermediate variety, variations between seasons and districts were considerable. Taking these variations into consideration, results indicate that the intermediate variety has little, if any, influence on the development of core-flush. Although dates of picking were all within the normal harvesting period for Granny Smith, it is possible that variance in maturity between orchards and differing time lag between picking and storing would account for some differences.

In one season where it was possible to compare fruit from trees which had received a dressing of borax of $\frac{1}{2}$ lb to a tree as against similar fruit when borax was not applied, there was a tendency for core-flush to increase with borax application. A similar result was noted by Phillips and Johnston (1943).

EXPERIMENTAL, 1946

Samples used in the previous two years showed wide and unexplained variations in the incidence of core-flush. It was considered possible that orchard management might play some part, as fruit had been collected from many different orchards. To test this point an attempt was made to select trees on the same rootstock in orchards under widely different types of management. Only two suitable blocks of trees could be found, these being Granny Smith on Northern Spy stocks. Orchard 1 was under good management, and the trees selected were superior to those of comparable age in Orchard 16 under poor management. Fruit from the only block of Granny Smith on Malling stocks was also included, as these stocks will be used for many future plantings in New Zealand. The trees on Malling stocks were in good condition and comparable with those on Northern Spy in Orchard 1. Four case samples were stored at 34°F. Picking and storage dates are shown in Table III. All fruit was examined between 24th and 31st of October.

RESULTS, 1946

Figures obtained from the 1946 experiment are given in Table III.

Picking and storage dates differed slightly for the two samples from trees on Northern Spy. Sample 16 was picked two days earlier and received four days extra delay than Sample 1. It is unlikely that the difference shown in the Table could be attributed to earlier picking, but longer delay before storing could account for some of it.

Results from trees on Malling stocks were similar to those from trees on Spy. When considered with the figures for 1944 it appears that the introduction of Malling stocks will not increase the incidence of core-flush.

Comparison of results obtained from samples marked 1 over the three years shows that least core-flush was recorded in 1946, the year of earliest picking, indicating again that core-flush is subject to seasonal variation, irrespective of harvesting date.

Table III. Nelson District 1946.
Percentages of Total and Commercially Significant Coreflush on Removal from Storage.

Orchard.	Rootstock.	Total Apples per Sample	Picking Date	Storage Date.	Period of Storage. (Days).	Per cent Total Coreflush	Per cent. Commercially Significant Coreflush.
1	Northern Spy	497	8/4	11/4	203	73	27
16	Northern Spy	499	6/4	13/4	194	87	63
	East Malling XII	463	8/4	11/4	202	59	25
3	East Malling XVI	453	8/4	11/4	201	69	24

Conclusions

Figures obtained from trees containing the susceptible varieties, Dunn's and Statesman, as intermediates showed no consistent increase in core-flush when compared with trees containing other intermediate varieties. Under commercial conditions differences in degree of core-flush can be expected between different orchards in any one season and from the same trees in successive seasons. There was no indication that the trees worked with any particular intermediate variety would give consistently higher or lower percentages of core-flush than trees budded directly on to the four root stocks listed. Thus in reworking existing trees to Granny Smith there is no need to consider the effect of intermediate variety on core-flush in future crops.

Acknowledgments

The author wishes to express his thanks to the numerous growers who supplied fruit samples, thus enabling this study to be made.

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REVIEWS

FIVE HUNDRED VARIETIES OF HERBAGE AND FODDER PLANTS.

C.A.B. Bull. No. 39, 1948

In this publication there has been collated information concerning a great number of varieties of herbage and fodder plants, including cereals and root crops, usable as animal fodder. The objects have been to give in as concise a form as possible many details regarding origin, adaptation, characteristics and use of varieties in various countries, together with the possibilities of obtaining supplies. Much of the information on crop varieties used in the feeding of farm stock has been obtained from specialists in many parts of the world, and is therefore of special interest to all research and advisory workers, to Plant Improvement Stations, and to distributors of seeds where the aim is improved varieties for field use.

There have been difficulties in nomenclature and unfortunately several important countries have been unable to make their contributions, but, nevertheless, this first effort to bring under one cover all relevant data concerning a great number of varieties of fodder plants will be of interest and value to many scientists, seed merchants and possibly growers of improved varieties of seeds.

An index containing references to published information on crop varieties has been added as a supplement.

L. W. G.

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This organization, formed by the Governments of the British Commonwealth, provides up-to-date information in the form of journals, technical communications, and bibliographies on all aspects of science and practice as applied to agriculture, horticulture and forestry. The following list comprises journals and other publications. Subscription rates are quoted after each. In certain cases (denoted by an asterisk) a 20 per cent. deduction is made for subscribers in the British Commonwealth who send their subscriptions direct:—

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DIET IN RELATION TO REPRODUCTION AND THE VIABILITY OF THE YOUNG. Part I. Rats and other laboratory animals. By F. C. Russell. 1948 6s.

The effects of dietary deficiencies on the reproductive performance of laboratory animals are briefly described, and data relating to the quantitative requirements for proximate principles, minerals, and vitamins during the processes of reproduction and rearing of the young are reviewed. An attempt has been made to correlate the results of such studies with the reproductive success obtained with stock diets subject to the limitations imposed by the operation of non-dietary variables which influence reproductive performances.

GROWTH SUBSTANCES AND THEIR PRACTICAL IMPORTANCE IN HORTICULTURE. By H. L. Pearse. 1948. 12s 6d.

A review of the actual and potential uses of synthetic growth substances in horticulture and the technical problems involved in their use.

INDEX TO HORTICULTURAL ABSTRACTS, Volumes XI–XV, 1941-1945. Compiler D. Aitkenhead. 1939. 35s.

A comprehensive subject and author index of the abstracts or notes occurring in these volumes of Horticultural Abstracts dealing with more than 10,000 original articles. Indispensable for full use of abstracts.

THE PRACTICE OF SOIL CONSERVATION IN THE BRITISH COLONIAL EMPIRE. By Sir Harold A. Tempany. 1949. 10s.

This is the first printed account to deal with colonial soil conservation in detail. The main conservation methods are classified, and their effectiveness in different territories is discussed. The control of grazing and livestock management and systems of colonial agriculture in relation to soil conservation are described. Chapters are devoted to the use of machinery and of aerial-photographic surveys, and an account is given of legislation relating to soil conservation that has been enacted in the colonial empire.

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THE METABOLISM AND TOXICITY OF CYANIDES AND CYANOGENETIC GLUCOSIDES IN SHEEP

1. ACTIVITY IN THE RUMEN.

By I. E. COOP and R. L. BLAKLEY
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Summary

(1) A sheep with permanent rumen fistula has been used to study the production of HCN from cyanogenetic glucosides and cyanogenetic plants in the rumen and the absorption of this HCN from the rumen.

(2) Absorption of HCN from the rumen is very rapid. On an average 75 per cent of dosed HCN is absorbed within 15 min.

(3) The hydrolysis of free glucoside and of glucoside in plant tissue in the rumen is rapid and can be complete in as short a time as 10–15 min.

(4) Hydrolysis in the rumen does not necessitate the presence of plant enzyme since the ruminal bacteria can hydrolyse cyanogenetic glucosides very rapidly.

(5) Peak production, concentration and absorption of HCN under most circumstances take place within a few minutes (less than 10 min.) of dosing free glucoside and within 10–20 min. of sheep eating cyanogenetic plant material.

(6) The conditions of the rumen affecting the rates of hydrolysis of glucosides and of absorption of HCN are discussed.

IN spite of extensive literature on cyanide poisoning, whether by pure cyanides or by the ingestion of plants containing cyanogenetic glucosides, considerable speculation still exists as to the precise mechanism of this poisoning and the manner in which cyanide is metabolized by the body. The first step in poisoning by free cyanide is obviously the absorption of HCN from the alimentary tract, but in the case of poisoning following ingestion of cyanogenetic plant material this absorption must be preceded by hydrolysis of the glucoside to yield the free HCN. In ruminants HCN must be absorbed from the rumen, as indicated by the following facts. The rapidity with which animals react to free cyanide administered per os indicates that absorption must be rapid and must take place before there is any possibility of significant amounts of the material passing further down the alimentary tract. This is confirmed by analysis of the ruminal and intestinal contents of sheep poisoned by cyanide. The appearance of symptoms of cyanide poisoning following ingestion of cyanogenetic plants is somewhat delayed, being seldom earlier than 5 min. after commencement of ingestion and may even be several hours after. Even so, the material is still almost certainly in the rumen and would not have had time to pass on in significant quantities to the reticulum, omasum and abomasum. This again is confirmed by chemical analysis of stomach contents. Auld (1) and van der Walt (2).

The hydrolysis of the glucoside is carried out by enzymes, normally present in the plant along with the glucoside, and it is believed that hydrolysis commences as soon as the plant is ingested. However, some plants contain glucoside only, some enzyme only, and some neither, even amongst different varieties in the same species (e.g., *Trifolium repens*).

In these cases it has always been assumed (van der Walt *loc. cit.*, Seddon and King (3)) that the plant containing glucoside only is not toxic unless another plant containing enzyme is also ingested, for otherwise the glucoside is not hydrolysed. Once released by hydrolysis the absorption of HCN can proceed in the same manner as if it had been administered as HCN or KCN. In theory the cyanogenetic plant should be as toxic as the equivalent amount of free HCN, but this has been shown by a number of investigators not to be true (see Part III). A number of factors can influence the release and absorption of HCN. The first and most important is the presence or absence of the enzyme. The second is the pH of the rumen, since under very acid or alkaline conditions the enzymic hydrolysis is inhibited or delayed (Auld, *loc. cit.*). In addition, under alkaline conditions, especially when chalk or lime is included in the diet, it has been postulated that HCN occurs as cyanide ions which cannot be absorbed through the ruminal wall (van der Walt, *loc. cit.*). Obviously other factors must also exert some influence, e.g., the physical state of the ingesta and ruminal contents and the nature of the previous diet.

In order to determine the part that all these factors play in the toxicity of cyanogenetic plants and to provide data for the interpretation of the result of dosing and feeding trials with free HCN, glucoside and with cyanogenetic plant material it was decided to investigate the fate of cyanide and cyanogenetic glucosides in the rumen of sheep. To this end a rumen-fistulated sheep was used for measuring the changes taking place in the rumen after introduction of free HCN and free glucoside into the rumen, and after ingestion of white clover, dried powdered white clover and linseed meal.

EXPERIMENTAL MATERIAL AND METHODS

The sheep with the rumen fistula was a typical three-year-old Romney wether. The operation was performed in August, 1946, according to the technique of Phillipson and Innes (4).

The cannula was made of perspex turned down so that an outer flange could be screwed down on to an inner flange within the rumen, thus holding in contact the wall of the rumen and the abdominal wall. The external end of the cannula tube was sealed by a cap screwing down on to the cannula. The dimensions of the cannula were: Length 6.5 cm., diameter of flanges 3.0 cm., external diameter of shaft 2.1 cm., internal diameter of shaft 1.6 cm.

During the year following the operation the sheep was used for experiments in digestion. In August, 1947, it was noticed that a complete seal which had been made between the ruminal wall and the abdominal wall was beginning to grow over the inner end of the cannula. The cannula came away easily, leaving an opening fistula. From then onwards a glass rod 11 cm. long and 1.0 cm. diameter with a flange at the outer end was used to maintain the fistula and to make it air-tight. The rod was held in position both by the fistula itself and by a string passed round the sheep and tied to the flange on the rod. The work to be described took place from September, 1947 to May, 1948. During this period the sheep was housed indoors except for occasional periods of a few days out on pasture. Its ration was chaffed lucerne hay plus chaffed timothy hay. At all times it remained in good health and vigour, increasing in weight from 140 lb. to 164 lb. over the period.

Potassium Cyanide

HCN was administered as KCN. The pH and buffering power of the rumen was such that this is equivalent to dosing with HCN. The KCN employed was usually in the form of a 0.1 M. solution (= 2.7 mg. HCN/ml).

Glucoside

Lotaustralin, the cyanogenetic glucoside of white clover, was used. The sample used was a crystalline solid, 90 per cent. pure, containing 90 mg. HCN per g. Lotaustralin is very stable to acid and alkali. Boiling with 2N. acid or alkali for 15 minutes produces less than 1 per cent. hydrolysis. Consequently there can be no hydrolysis of the glucoside by acid or alkali under the conditions of experimentation to be described.

Enzyme

Linamarase, the enzyme hydrolysing lotaustralin, and linamarin was prepared from linseed by the method described by Coop (5) taking it as far as 'Preparation I'.

pH Measurement

A glass electrode pH meter made by the Cambridge Instrument Co. was used.

Determination of HCN

It was essential in this work to use a method of analysis which was rapid, sensitive, and specific. The only method which reasonably fulfils these three requirements is that devised by Gettler and Goldbaum (6). This relies on the Prussian Blue test, which is developed by aeration of the HCN solution and passage of the gases through filter paper impregnated with ferrous sulphate and sodium hydroxide. After aeration the excess of iron hydroxide is dissolved out with hydrochloric acid. Standard Prussian blue stains are prepared with which to compare the unknowns. The method is specific and no known substances interfere with it. It is specific and no known substances interfere with it. It is also rapid as the aeration takes only five minutes. This is an important advantage over the methods such as that of van der Walt and other investigators, for where samples are being taken at intervals of every 10-20 minutes a reaction can be followed as it proceeds, since a rough estimate of the HCN value can be made immediately after placing the test paper in the acid. At a later stage one can analyse the duplicate sample and estimate all the values accurately by comparison of the stains with the standards. As regards sensitivity the method as used in this study covers the range 0.5–20.0 μg. (micrograms). Gettler and Goldbaum claim an accuracy of 0.1 μg. within this range, but this accuracy was seldom achieved in our work. There was often a considerable variation (up to 15 per cent.) between duplicate stains, and in this case it was necessary to make three estimations per sample. The deepest stain was in all cases taken as the correct one, since estimations of standard solutions showed negative errors only. With these precautions it is estimated that the error is of the order of 10 per cent. when the estimation is done in duplicate and 5–10 per cent. when done in quadruplicate.

Estimations on pure KCN solutions at room temperature were subject to errors of the magnitude described. These must be attributed to irregularities in the test papers and to errors in reading. Gettler and Goldbaum dried the test papers, both after ferrous sulphate and after sodium hydroxide impregnation, for an unspecified period in air. Our own experience showed that more satisfactory results are obtained by drying the papers at 60–70°c. by suspending them in air vertically over a hot plate until thoroughly dry. This avoids both the formation of a hard crust of sodium carbonate, which makes it difficult to draw the gases through the paper, and too much oxidation to the ferric state. For the same reason the papers should be stored immediately in an air-tight vessel, where they may be kept for about a week. Using either procedure for drying the resulting papers are far from uniform, and irregularities in drying, oxidation, pore size, and carbonate formation must account for much of the error of the method.

Even if these errors could be eliminated, it is not possible to compare two stains by the unaided eye with an accuracy greater than 5 per cent. Hence the errors inherent in the method preclude an accuracy greater than this.

A further improvement was effected by replacing the rubber bands in the apparatus of Gettler and Goldbaum by perspex clamps. This is an important point, since if the glass flanges are not pressed tightly and uniformly together the blue stain forms in one small region of the paper. After a few days the test paper becomes coated with carbonate, making it difficult to draw air through the paper, and any small leakage of air causes bad distribution of colour over the paper.

METHOD OF DOSING AND SAMPLING

The apparatus used consisted of a piece of glass tubing about 25 cm. long and 1 cm. external diameter attached by means of a short piece of pressure tubing to a splash head bulb which in turn was attached to a 100 ml. syringe. The solution to be dosed was sucked up into the bulb. The rod plugging the fistula was then removed, the 25 cm. tube introduced into the rumen, and the liquid injected by closing the syringe. Immediately after withdrawing the tube the fistula was plugged again. In some cases the solution was given through the fistula by means of a drenching gun.

At varying intervals after giving the dose, samples were taken from the rumen. This was performed, using the above apparatus, by introducing the tube into the rumen, sucking the fluid slowly into the bulb, and then forcing it into a sampling tube. Two separate samples were taken each time. Since the dose and the sample were administered and withdrawn through the same opening (fistula) and with the same apparatus, it was necessary to exercise considerable care after dosing with KCN to avoid contamination of the sample.

It was often difficult to obtain a sample if the sheep had just previously eaten to capacity, especially on the chaffed hay usually provided. In such an event it was necessary to wait for the rumen to contract, when any fluid from the rumen and reticulum was thrown up on top of the solid ruminal mass. Such a sample was not a true sample of the rumen, but was representative of the fluids draining and circulating through the rumen. Because of this difficulty most experiments were conducted while the rumen was reasonably fluid.

If the sheep was fed in the evening the rumen was usually in a satisfactory state next morning and a light feed could even be given before and during the experiment.

The sample tubes were 15 ml. centrifuge tubes, into each of which 1 ml. of 0.5 N. NaOH had been introduced. The sample of ruminal fluid varying from 5–12 ml. was then added from the sampling apparatus. The tubes were immediately corked and shaken. The NaOH by making the fluid alkaline (pH 9–10) prevented loss of HCN and also, after glucoside dosing, any hydrolysis of the glucoside and consequent increase in HCN after sampling. Thorough tests showed that this technique was effective and that within experimental error no loss of HCN occurred. The level of the fluid in each tube was then marked so that the volume of sample taken could subsequently be measured. HCN estimations were then made on these samples. Where the HCN content was relatively high the sample was diluted to bring it within the range of estimation. Before an aliquot was taken from a diluted sample it was necessary to acidify because the NaOH makes the ruminal fluid so mucilaginous that it does not disperse evenly, even when shaken with water.

RESULTS

Rate of Absorption of HCN from Rumen

The rate of absorption of HCN, dosed as KCN, was measured by the technique described. The assumption is made that the disappearance of HCN from the rumen is due to absorption from the rumen and to no other cause. The disappearance is so rapid that it cannot be due to passage down the intestinal tract or to detoxication within the rumen (see Part II). The results obtained are set out in Table I.

TABLE I. CONCENTRATION OF HCN IN RUMEN AT INTERVALS AFTER DOSING, CONCENTRATION EXPRESSED AS μG ML.

Experiment No	1	2	3	4	5	6	7	8
Dose (as HCN)	40 mg.	72 mg.	72 mg	97 mg.	100 mg	110 mg.	128 mg.	150 mg.
pH of rumen	5.54	8.45	6 76	---	8.02	6.62	6.62	5.52
Time (minutes)								
0	(13)	(24)	(24)	(30)	(33)	(36)	(43)	(50)
5	-	8.7	6.7	10.2	-	-	—	- -
15	3.9	4.8	2.2	1.8	7.8	8.6	8.5	25
30	2.9	3.0	1.7	0.5	3.5	2.5	1.2	4.3
45	2.3	1.5	0.9	0.0	2.4	1.3	0.8	3.2
60	1.9				1.8			2.5
120	0.8				1.4			1.3
180	0.1							0.7

The concentrations at 0 minutes are the theoretical initial values, assuming the volume of the rumen to be 3 litres and assuming complete mixing. The concentrations at the intervals stated were those measured. Sometimes the time intervals were not strictly those given, in which case the concentrations were obtained by interpolation, but in no case was the smoothing of curves done. It was found that concentrations measured between 0–10 min. were unreliable in that considerable variations existed between duplicate samples. This was undoubtedly due to incomplete distribution of the dosed cyanide throughout the rumen, and the resultant possibility of striking a pocket of high HCN concentration. As a result the first sample was usually taken 15 min. after dosing.

The experiments took place at various times over a period of eight months. With the exception of Experiments 2 and 5, the sheep had been fed normally and had access to food during the experiments. In the case of Experiments 7 and 8, fairly severe symptoms of cyanide poisoning were manifest. In order to test whether either starvation or a high pH affected the rate of absorption, the sheep was starved for 48 hours before dosing in Experiments 2 and 5, whereas in the other experiments it had been dosed immediately after a full feed. Several observations emerge from these results:—

(i) The rate of absorption of HCN is extremely rapid. On an average, about 75 per cent. is absorbed within the first 15 min. and over 90 per cent. within half an hour of dosing.

(ii) A variation between experiments, though not great, does exist and cannot be attributed with reliability to any definite cause. There is no significant correlation with pH or degree of feeding. Part of it is probably due to experimental error in sampling and estimation, but it is unlikely that this would account for more than a small fraction of the total.

(iii) The effect of starvation and alkaline conditions (Experiments 2 and 5) in the rumen does possibly reduce the rate of absorption slightly, but it is within the normal variation of normal sheep. Certainly the effect, if any, is not marked.

(iv) The rate of disappearance of HCN from the rumen is so rapid that absorption directly from the rumen must be the means of removal.

(v) Owing to the variation between experiments, it is difficult to estimate a true rate of absorption. However, taking the average of the whole eight experiments, the rate of absorption in the first 15 min. at an average concentration of 20 μg./ml. is about 1.7 μg./ml. per minute. Over the period from 15–45 min. at an average concentration of 3–4 μg./ml. the rate of absorption is about 0.1–0.15 μg./ml. per min.

The Hydrolysis of Cyanogenetic Glucosides by ruminal microflora

Before studying the effect of dosing with glucoside, it was shown that microflora of the rumen could hydrolyse the glucoside with release of HCN. Preliminary experiments of introducing lotaustralin into the rumen showed that HCN could be detected in the ruminal contents several minutes after dosing. This could be due to HCN already present in the diet, to enzyme present in the diet, or to bacterial hydrolysis of the glucoside. Experiments showed that the lucerne hay which formed part of the diet did contain extremely small amounts of both cyanogenetic glucoside (0.5 μg. HCN/g.) and enzyme, but such amounts were quite incapable of accounting for the rate of hydrolysis of the lotaustralin. The diet of the sheep was nevertheless changed to one of turnips, chou moellier, and crushed oats, all of which were shown *in vitro* to be totally devoid of either glucoside or enzyme. After a week on this diet the results were precisely the same.

Ruminal liquor was then taken and its activity determined by its rate of hydrolysis of lotaustralin *in vitro* (in Conway units). Some of the liquor was at the same time centrifuged. Centrifuging at 500 r.p.m. for 1½ min. threw down material containing most of the activity while the supernatant liquid after centrifuging at 2,500 r.p.m. for 10 min. contained only a small percentage of the original activity.

Finally cultures of the ruminal microflora were made in liquid and solid media containing tryptose and lotaustralin. Two organisms were found to grow well under aerobic conditions: a Gram positive diplococcus, and a Gram negative bacillus. Cultures of these organisms readily hydrolysed lotaustralin. These facts, together with the data to be presented in the remainder of this paper, leave no doubt that the ruminal microflora are capable of releasing HCN from cyanogenetic glucosides.

The Rate of Hydrolysis of Lotaustralin by ruminal microflora in vitro

The rate of hydrolysis of lotaustralin by ruminal microflora was measured by withdrawing a sample of rumen liquor and rapidly transferring this to an incubator where any sediment and large food particles were allowed to settle. The supernatant liquid was decanted off into the reaction tubes. Glucoside was then added at a rate varying from ½–1 ml. of solution per 10 ml. of ruminal liquor. The time interval between taking the sample and the commencement of the reaction was less than 5 min. The temperature was maintained at 37°c. The reaction vessel was a test-tube, tightly corked, from which 1 ml. samples were taken very rapidly at intervals for analysis. At first the incubation was carried out under a layer of paraffin oil to prevent loss of HCN and to maintain an aerobic condition, but this proved to be unnecessary. The simple technique described might be criticized on the grounds that there would be loss of HCN each time the tube was opened and a sample taken. Using glucoside of known strength it was found that within experimental error (± 5 per cent.) there was no loss. The reaction is certainly aerobic, whereas in the rumen it is anaerobic, but this likewise had no significant effect, possibly because the reaction is fast and completed before aerobic conditions have time to modify the bacterial population and activity. In the interests of rapid working this crude but effective method was used in all incubation experiments.

Some typical results obtained are given in Table II:—

TABLE II. RATE OF HYDROLYSIS OF LOTAUSTRALIN

Date	Concn. of Glucoside as HCN in Reaction Vessel (μg. ml.)	pH	Time to 50 per cent Hydrolysis (Minutes)
22/9/47	17	6.8	16
24/9/47	17		22
27/4/48	30	6.0	10
5/5/48	30	7.4	10
18/8/48	30	7.3	4

These rates show that the hydrolysis is rapid. In the sheep itself the rate must be even more rapid, since in the *in vitro* work there is dilution of about 10 per cent. and some chilling (about 7°c.) in the few minutes following withdrawal of the sample. The concentrations of glucoside in these reactions are the same order as those used in the subsequent *in vivo* experiments. The results indicate that the maximum rate of production of HCN should occur at an interval not greater than about 15 min after administration of glucoside.

The optimum pH of the bacterial reaction was measured by the same technique. The sample withdrawn was brought to the desired pH by the addition of either dilute acetic acid or caustic soda. The rates of hydrolysis were then measured and corrected for the slight dilution by acid or alkali. This was repeated at a subsequent date, but the two determinations agreed well. The pH curve so obtained is given in Fig. 1.

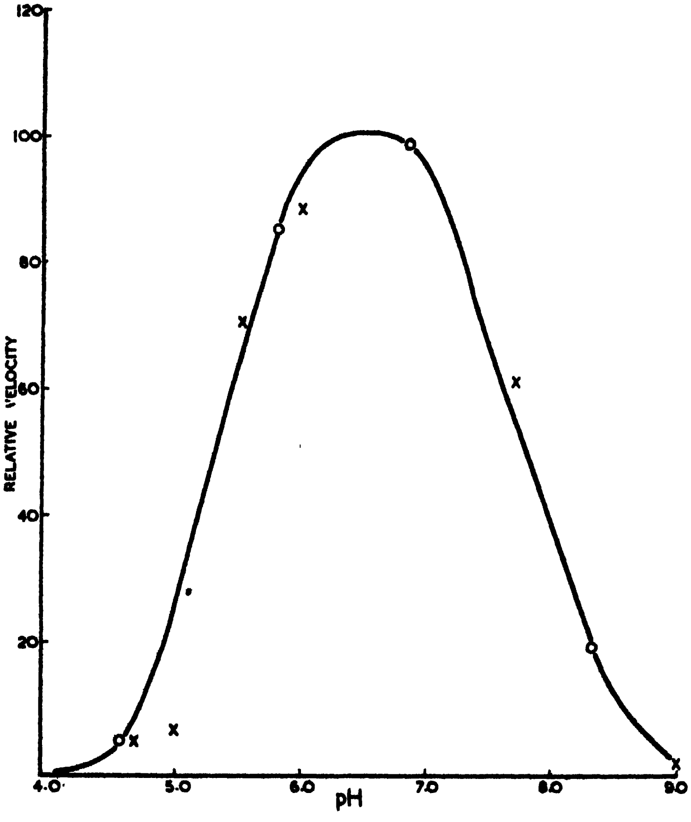

FIG. 1

By comparison with the pH curve of the enzyme linamarase (Coop, *loc. cit.*) the bacterial hydrolysis is much less tolerant to acid conditions. Whereas linamarase possesses 90 per cent. of its maximum activity at pH 5.0 and 60 per cent. at pH 4.0 the bacterial activity is reduced to a low value even at pH 5.0. This indicates that the hydrolysis in the rumen is a function not only of the enzyme system within each bacterium, but is also a function of the medium as an environment for bacterial growth and metabolic activity. The normal range of the pH found in the rumen of sheep is approximately 5.5–7.5 which coincides with the conditions of optimum hydrolysis by ruminal fluid. On the alkaline side, the activity falls to below 10 per cent. of the maximum at a pH of 8.6. This fact will be referred to later, in Part III, for it is possible to delay the hydrolysis in the sheep by inducing an alkaline reaction in the rumen.

The Effect of Salts and Sugar on Rate of Hydrolysis in vitro

At various times it has been stated that the administration of glucose, sulphur, sodium thiosulphate, ferrous sulphate, and other substances is beneficial in the treatment of cyanide poisoning (van der Walt, *loc cit.*). The effect of these substances on the hydrolysis of glucosides was measured *in vitro.* 10 ml. of rumen liquor was incubated for 15 min. with each of the following substances and then 1 ml. of glucoside solution added to give a concentration of .15 mg./ml. (equivalent to 15 µg. HCN/ml.). The following results were obtained (concentrations expressed as the concentration in the reaction vessel):—

(i) Glucose 50 mg./ml. Velocity reduced to less than 5 per cent.

(ii) Glucose 10 mg./ml. Velocity reduced to approx. 10 per cent.

(iii) Glucose 2 mg./ml. Velocity reduced to approx. 50 per cent.

(iv) Sulphur (Flowers) 1 mg./ml. Velocity reduced to approx. 75 per cent.

(v) Sodium Sulphide 1 mg./ml. Velocity reduced to approx. 70 per cent.

(vi) Sodium Thiosulphate 1 mg./ml. Velocity reduced to approx. 80 per cent.

(vii) Sodium Nitrate 1 mg./ml. Velocity reduced to approx. 75 per cent.

(viii) Ferrous Sulphate (hydrated) 1 mg./ml. Velocity reduced to approx. 50 per cent.

It will be noticed that all reduced the rate of hydrolysis, the most significant being glucose. The salts and sulphur in the concentrations used certainly reduced the rate to some extent, and whether this was due to a depressing effect on bacterial activity or due to their entering into some detoxication mechanism is not known. The effect of glucose is noteworthy. A dose of 30 g. of glucose (equivalent to 10 mg./ml. in a 3-litre rumen) reduces the rate of hydrolisis to 10 per cent. of its original value. No doubt this is the cause of the antidotal value of glucose. van der Walt (*loc. cit.*) and Worden (7), explain the influence of glucose as combining with HCN to form the cyanhydrin. This is unlikely, seeing that the cyanhydrin is already an intermediate compound in the hydrolysis of glucoside to yield glucose, HCN and another constituent. A more likely explanation is that the readily available glucose competitively inhibits attack on the glucoside by the bacteria. When there is ample glucose there is no need to utilise glucoside. Further proof that glucose does not hold the HCN as cyanhydrin was obtained by aerating solutions containing 3 µg./ml. and 1 µg./ml. of HCN at 37°c. and at a pH of 6.5, alone, and in the presence of 2 per cent. glucose. The glucose did not decrease the rate at which the HCN was given off by aeration. The HCN plus glucose was then incubated for 40 min. both with and without ruminal liquor. This did not have any effect either, though incubation for 24 hours did procure a significant reduction. The conclusion must be reached therefore that glucose does not affect the absorption of HCN in the rumen but influences its rate of production from the glucoside.

Rate of Hydrolysis of Glucoside and of Absorption of HCN in vivo

Having shown that the microflora of the rumen can bring about the Hydrolysis of lotaustralin with release of HCN at such a rate that the addition of enzyme preparations would not be of any advantage, all administrations of glucosides were carried out in the complete absence of enzyme.

Glucoside solution was introduced into the rumen through the fistula, and ruminal samples taken at intervals for determination of HCN present. In the case of HCN administration samples taken for HCN estimation in the first 10–15 min. tended to be unreliable, but where one is measuring HCN produced from a substance containing no free HCN it would be expected that the values would be more consistent and more reliable. This was found to be true, though there were still irregularities due to incomplete mixing in the rumen.

The results obtained at various times over a period of 8 months are given in Table III. In every case the dose was the same, 1.1g. of 90 per cent. lotaustralin (equivalent to 100 mg. HCN).

TABLE III. CONCENTRATION OF HCN IN RUMEN EXPRESSED AS μG. ML. AT INTERVALS AFTER DOSING WITH LOTAUSTRALIN

Experiment No. Dose (as HCN) pH of rumen	9 100 mg	10 100 mg. 6.10	11 100 mg 6.32	12 100 mg 7.12	13 100 mg 6.54	14 100 mg 6.82	15 100 mg. 8.12
Time (Minutes)							
5	6.7	-	-	7.7	20	6.5	
10	3.1	9.7		7.2	15		
15	1.3	3.0	6.1	4.8	5		6.2
30	0.2	0.6	3.9			3.4	5.0
45	-	0.2	2.7			1.9	
60	0.0	0.1	2.5				4.3
120			0.1				3.0
180							2.1

The values quoted are those actually obtained, and are not taken from smoothed curves. Assuming a ruminal volume of 3 litres, the theoretical HCN concentration for complete hydrolysis and without any absorption of HCN would be 33 μg./ml. The actual concentration in the rumen is the difference between the rate of production of HCN by hydrolysis and the rate of absorption of the HCN produced. The former can vary according to the concentration and activity of the bacteria, while the latter varies according to concentration in a manner illustrated in Table I.

Several observations can be made on these figures:

(i) The rate of hydrolysis is very rapid. In the case of Experiment 13, apparent hydrolysis had proceeded to the extent of 60 per cent. within 5 min. and in Experiments 9, 12, and 14 to the extent of 20–25 per cent. The actual amount of hydrolysis would be even greater since no allowance for absorption has been made.

Excluding Experiment 15, which was abnormal (see below) hydrolysis must for all practical purposes be complete at times, varying from 10–15 min. in the case of Experiment 9 to 1 hour in Experiment 11.

(ii) Considerable variation exists in Experiments 9–14 between the concentrations at any given time. This could not be explained in terms of pH, amount of feed eaten, and consistency of ruminal contents. The variation is probably due to varying concentrations and activities of the ruminal bacteria which in turn depend upon the distribution of food intake over the previous 24 hours. Unfortunately, the latter was not recorded at the time.

(iii) Experiment 15 was specifically designed to reduce the bacterial activity. The sheep had been starved for 60 hours at the time of dosing, the pH was high (8.12) and the ruminal contents were very fluid. In the morning before dosing, KCN had been administered to determine whether the starvation affected rate of absorption of HCN (Experiment 5, Table I), and it was found that the latter was not markedly slower than normal. The data in Experiment 15 show that the rate of hydrolysis is definitely reduced. Initially at least 20 per cent. hydrolysis must have occurred in the first 15 min. but the maintenance of a concentration of 2–3 μg./ml. at 2–3 hours shows that there is still significant hydrolysis taking place even then. The reduction in rate may be due either to a reduction in the concentration of bacteria or to pH alone or both.

Hydrolysis of Lotaustralin in Wet Clover

The experiments described above dealt with the glucoside in a free state. In practice it is the fate of the cyanogenetic glucosides in the plant as eaten that is of greatest interest. The sheep was therefore fed cyanogenetic plant material and the concentration of HCN in the rumen measured.. Parallel experiments were conducted *in vitro* to determine the rate of hydrolysis by ruminal fluid.

The first plant material given to the sheep was white clover. The strain used contained both glucoside and enzyme. As it was considered important to know the rate of eating the sheep was not put out on to a clover paddock, but the clover was cut and fed indoors. Details of the experiments were as follows:—

(i) Experiment 16. The clover was cut with a scythe, gathered up and fed to the sheep within 10 min. The clover itself was rather damp. The HCN content was 0.008 per cent. pH of rumen = 6.17. The sheep ate 650g. in 45 min., after which the clover was removed.

(ii) Experiment 17. The clover was dry, the day being fine and sunny. It was fed 10 min. after cutting. The sheep ate 800g. in 15 min., after which the clover was removed. HCN content was 0.008 per cent., but the pH of the rumen was not recorded.

(iii) Experiment 18. Same as for Experiment 17. Sheep ate 850g. in 15 min.

The results of these experiments are given in Fig. 2. In this graph are also plotted the results of *in vitro* experiments in which clover was incubated to determine the rate of HCN production. The clover was picked from the same plot, but unfortunately not on the same days as the previous *in vivo* experiments, so that the results are not strictly comparable. The clover was bruised in a mortar to stimulate the physical state of ingested clover in the rumen and was then incubated both with ruminal liquor and with water (3 g. clover+12 ml. ruminal liquor or water). The time from picking to incubation was 15 min.

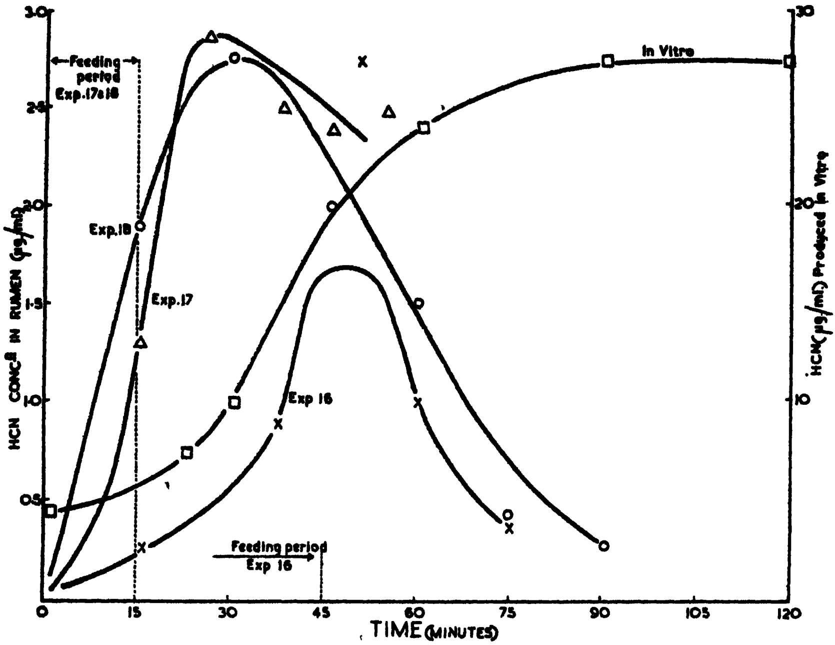

FIG. 2

The special features of these results are as follows:—

(i) The *in vitro* hydrolysis is considerably slower than that of free glucoside, the time to half decomposition being of the order of 40 min. as against 10–20 min. for free glucoside. Furthermore, the enzyme present in the clover must account for most of the HCN released, even in the presence of ruminal liquor, since addition of the latter did not accelerate the production of HCN.

(ii) Though the eating of 800 g. of clover is equivalent to 64 mg. HCN at no time did the HCN level of the rumen rise above 3 μg./ml. This is undoubtedly due to the slower rate of hydrolysis so that the HCN is absorbed almost as fast as it is produced.

(iii) Peak HCN concentration is reached fairly consistently about 20 min. from the mid-point in time of the feeding period. This indicates that the *in vitro* reaction is slower than the *in vivo,* which is to be expected.

Hydrolysis of Lotaustralin in Dried Clover

A sample of dried clover was made available from the Plant Chemistry Laboratory, Palmerston North. This clover contained glucoside, equivalent to 0.104 per cent. HCN, but it contained no enzyme at all. Incubation of the clover alone produced no HCN. Unfortunately, in anticipation of introducing the clover through the fistula the dried material had been ground. As it subsequently proved difficult to introduce through the narrow hole of the fistula at a sufficiently rapid rate it had to be fed to the sheep. Being a powder, it was difficult to eat, but with some training and the inclusion of 33 per cent. crushed oats, the sheep was coaxed to eat some.

(i) Experiment 19. The sheep ate 160 g. over a period of 96 min. The HCN level of the rumen remained fairly constant between 0.8–1.0 μg./ml. during the feeding and fell to less than 0.1 μg./ml. 40 min. after taking the clover away.

(ii) Experiment 20. The sheep ate 66 g. (= 69 mg. HCN) within 15 min., after which the clover was removed. pH of rumen was 6.0.

(iii) Experiment 21. The sheep ate 50 g. (= 52 mg. HCN) in 15 min. and the feed was then removed. pH was not recorded.

Simultaneously with the *in vivo* experiments, Nos. 20 and 21, *in vitro* incubations were made, using 15 ml. ruminal liquor plus 0.5 g. dried clover, this proportion being of the same order as that in the rumen. The results of Experiments 20 and 21 and of the parallel *in vitro* experiments are given in Fig. 3. As it was thought that the powdering of the dried clover might be responsible for the rapid rate of hydrolysis, the dust was sieved out and the coarse fraction not passing a 40 mesh sieve was compared with the original material. This fractionation did not affect the rate of hydrolysis, the only difference being that the coarse fraction was rather lower in HCN than the original. In Fig. 3 the *in vitro* curves are those obtained using the coarse material.

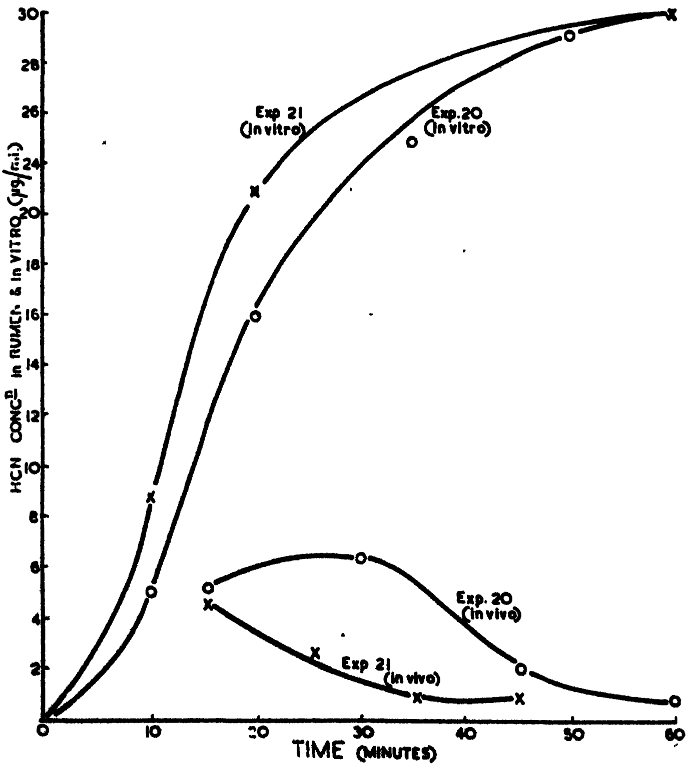

FIG. 3

From Fig. 3 it will be seen that:

(i) The time to half decomposition in the *in vitro* incubations is 17–19 min. Even in the complete absence of enzyme the bacterial hydrolysis is very rapid.

(ii) Peak HCN concentration *in vivo* is reached within 10–25 min. from the commencement of the feeding period and is reached earlier on the day in which the *in vitro* hydrolysis is more rapid (Experiment 21).

Hydrolysis of Linamarin in Linseed Meal

The only linseed preparation available was in the form of commercial linseed nuts, which were low in HCN content (0.030 per cent.) and were lacking in enzyme activity.

In vitro incubation of crushed linseed nuts gave zero HCN due to absence of active enzyme, but when incubated with ruminal liquor at the rate of 0.5 g. linseed to 15 ml. of liquor the time to half-hydrolysis was 15 min. The half-hydrolysis time, using free lotaustralin and the same ruminal liquor was 10 min.

Experiment 22. Simultaneously with the incubation the sheep was given uncrushed linseed nuts, eating 380 g. in 15 min., after which the linseed was removed. The HCN content of the rumen was as follows, the times quoted being from commencement of feeding:—

1.6 μg./ml. at 15 min., rising to a maximum of 2.6 μg./ml. at 30 min., and falling to 0.5 μg./ml. at 90 min. The pH was 7.4.

On another occasion it ate 622 g. in 15 min. and the HCN content of the rumen fell consistently from 1.5 μg./ml. at 15 min. to 0.3 μg./ml. at 60 min. The pH was not recorded, and on this occasion no incubation was made.

These observations prove that:—

(i) Linamarin is rapidly hydrolysed by rumen microflora;

(ii) Assuming the rate of hydrolysis of linamarin and lotaustralin to be the same, which is likely on account of their similarity in constitution, the rate of hydrolysis of linamarin in crushed linseed is about two-thirds as rapid as that of the free glucoside (half time 15:10 min.).

(iii) The HCN in the rumen remains at a low level. As the nuts fed to the sheep were uncrushed, the comparison between *in vitro* and *in vivo* cannot be carried far.

Hydrolysis of Amygdalin in the Rumen

By the previously described *in vitro* technique it was shown that the bacteria hydrolyse amygdalin readily, the rate of hydrolysis being about 60–70 per cent. as fast with lotaustralin. As further confirmation of this hydrolysis, the sheep was dosed per fistula with 1.7 g. amygdalin equivalent to 100 mg. HCN. The pH of the rumen was more alkaline than usual, viz., 7.62. The values of HCN concentration in the rumen were as follows:—10 min., 3.9 μg./ml.; 30 min., 1.7μg./ml.; 60 min. 0.2 μg./ml.; 90 min., 0.1 μg./ml. These figures are comparable with those obtained by a similar dose of lotaustralin (Table II).

pH and Buffering Power of Ruminal Liquor

One would expect the fluids of the rumen to possess considerable buffering power, and this proved to be correct. Thus the addition of 1 ml. 0.5N. NaOH to 10 ml. rumen liquor usually raised the pH to 9–10, but not beyond. The buffering power is greatly influenced by the state of the ruminal contents, the rumen of a starved sheep having a much lower power. Thus the addition of 1 ml. of 0.1N. NaOH per 10 ml. of rumen liquor changed the pH by only 0.37 in a well-fed sheep and by 2.0 in a starved sheep with very fluid ruminal contents. On one occasion when lime was added it took 2 mg. CaO/ml. to shift the pH from 6.5 to 9.5. In an endeavour to produce alkaline conditions in the rumen without administering caustic alkalis the sheep was given powdered chalk. Overnight it ate 80 g. $CaCO_3$ with its food. The following morning it ate a further 40 g. with hay and 20 g. was introduced through the fistula at 11 a.m. An hour later the pH was only 6.62.

These observations are of interest, for throughout the literature references are made to acid or alkali-producing diets having an effect on HCN liberation. As the pH is not measured and quoted it is believed that such statements can be misleading. In 18 months of measuring ruminal pH's on a variety of diets and under a variety of conditions, the extreme values obtained were 5.5 and 8.5.

Fig. 1 shows that the rate of hydrolysis of glucoside by ruminal bacteria is reduced to a low level in the region of pH 8.0–9.0. Some considerable reduction in hydrolysis can therefore be expected under practical conditions at the more alkaline pH's. On the other hand, some authors (van der Walt, *loc. cit.*) also contend that these alkaline conditions decrease the rate of absorption of HCN since some of the cyanide will be present as CN ions. This is clearly of little sigificance since the pK for HCN is 9.14 and the pH would have to hise above 9.0 before much cyanide was present in the ionic form. This has been borne out in any case by the results quoted in Table I.

DISCUSSION

The practical implications of the observations made in this paper will be discussed in Part III.

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SHEEP DIPPING TRIALS WITH DERRIS, BENTONITE SULPHUR, D.D.T., AND BENZENE HEXACHLORIDE

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Summary

Dipping trials have been conducted with derris, bentonite-sulphur, D.D.T. and benzene hexachloride against the sheep ked and the sheep louse. Derris has been shown to be very effective against keds but its control of lice has not been satisfactory Bentonite-sulphur has proved to be unsatisfactory for the control of both keds and lice. D.D.T. has been shown to be capable of controlling keds but, like Derris, cannot be relied upon to control lice. The best material tested was benzene hexachloride, which controlled keds with the same efficiency as derris or D.D.T., but in addition, as far as the experiments have gone, has shown promise of controlling lice The practical limitations and possibilities of the use of these materials in sheep dipping are discussed.

Introduction

In recent years attempts have been made to produce sheep dips other than the standard arsenic and phenolic types. The latter are far from ideal in that they are poisonous and are not completely effective. Whilst, for instance, the majority kill all adult keds at dipping, the materials do not last long enough in the fleece to kill the pupae when they hatch, and so provide little measure of control. Considerable improvement in effectiveness was made when the so-called 'quick-acting' types came into use. In these an extract of Derris root, containing the active principle, rotenone, was incorporated and this gave a greater measure of ked control for it killed all adults and remained in the fleece for sufficient time to be toxic to newly hatched pupae. Even so, these commercial dips are not entirely satisfactory. They are poisonous to sheep and humans, they are expensive and their period of toxicity in the fleece is short. Thus any dip which is non-poisonous, is cheap and confers a longer period of toxicity, is to be preferred. In the ideal this period of persistence of toxicity needs to be of the order of 12 months, so that reinfestation from 'stragglers' or unmustered sheep cannot take place from one shearing or dipping until the next, one year later. In such an event external parasites should be capable of eradication with comparative ease even on our rough hill country.

In 1941 Morrison and McLeod (1) first suggested the use of derris powder alone as a dipping material for the control of keds (*Melophagus ovinus*) and body lice (*Bovicola ovis*). Their experiments showed that a suspension of derris in water was highly toxic to keds, killing all live ones and remaining in the fleece long enough to kill all young keds emerging from pupae. Thus a single dipping was sufficient to eradicate keds completely from a flock provided it could be isolated from undipped sheep.

Though the period of persistence of toxicity was not long (of the order of 4-6 weeks) the dip had the advantage of being harmless to sheep and humans and of being very cheap. At the time it was thought that derris was also equally effective against lice, though the number of experiments on lice was fewer. Since the publication of the original work many thousands of sheep have been dipped in derris. The field experience of derris dipping together with further experimental work to be described later, has indicated two short-comings of derris powder dips. Firstly, in a considerable number of cases, a temporary lameness has been reported by McLean (2) following dipping in derris. Secondly, our own work has subsequently shown that derris, while killing all adult lice, cannot be relied upon to control them completely. This present paper reports the results of further work with derris, particularly against lice.

In search of new dipping materials, reports from U.S.A. (3) indicated that bentonite-sulphur was toxic to keds and lice and furthermore gave a much longer period of toxicity in the fleece than any material so far examined. It was claimed that this period was about 300 days. It was decided to test these claims.

In 1946 the new synthetic insecticides D.D.T. and benzene hexachloride became available in sufficient amounts to test their effect on keds and lice. In the meantime experiments with D.D.T. and benzene hexachloride have been reported from overseas. Heath (4) showed that D.D.T. at a concentration of 0.5 per cent. in the dip killed all the keds and prevented reinfestation for a period of at least five weeks. Mitchell and Heath (5) tested D.D.T. against the sheep tick (*Ixodes ricinus*) and found it to be fairly toxic though not completely satisfactory. They also prepared and tested many different types of D.D.T. emulsion. Later, Heath (6) tried lower concentrations of both D.D.T. and benzene hexachloride against keds, the concentrations of the crude insecticides being 0.5 per cent., 0.25 per cent. and 0.125 per cent. The initial kill was complete in all cases and the period of toxicity was determined by subjecting the sheep to reinfestation. The benzene hexachloride dips remained toxic until the conclusion of the benzene hexachloride experiments 10 weeks after dipping. The D.D.T. dips were toxic 8 weeks after dipping but were no longer toxic in the fleece 14 weeks after dipping. Heath concludes that either D.D.T. or benzene hexachloride at 0.125 per cent. concentration is sufficient to eradicate keds provided isolation can be maintained. In U.S.A., Rude and Parish (7) dipped 5,000 sheep and goats in a 0.2 per cent. D.D.T. dip and showed that they remained free of keds for 13 weeks after dipping. In Australia D.D.T. has given promising results (private communication N.P.H. Graham) where good control of keds was achieved with 0.1 per cent. D.D.T. The concentration fell considerably as the sheep were put through the dip. With a 1,000 gal. power spray dip the concentration fell from 0.08 per cent. to 0.05 after 1,000 sheep had been dipped. Nicol (8) obtained complete control of keds at a concentration of 0.2 per cent. and there was also some ' stripping out ' of the D.D.T.

In the dipping trials reported in this present paper much lower concentrations of D.D.T. and benzene hexachloride were used and their effectiveness against both keds and lice was investigated. No attempt has been made to determine the effect of these dips in blowfly strike prevention, though it is recognized that this feature may also be of importance.

Experimental Technique

The technique used was the same as that of Morrison and McLeod (1). The sheep were dipped individually in a small portable tank holding 50 gal. of dip, the sheep being held in the bath for 30 seconds approximately. After dipping, the sheep of each group were isolated in double-fenced pens. Keds and lice were counted before dipping, about 5-10 days after dipping and thereafter at monthly intervals. Keds and pupae were easy to count but lice were often difficult to find. At least 20 minutes' examination of each sheep was made before declaring it free of lice.

In recording the degree of infestation an arbitrary classification has been made into heavy, medium and light when the ked or louse populations were >30, 10-30 and 1-10 respectively. These have been recorded in the tables as + + +, + + and + respectively whilst complete absence is designated thus --. The sheep used were sometimes of mixed breeds and type. In as far as breed type influences dipping, this has been recorded in the tables as CW - coarse woolled, such as Romney English Leicester and Border Leicester, and as FW - fine woolled such as Corriedale and halfbred. It was recognized that the length of wool on the sheep at dipping influences the amount of dip retained by the fleece and may therefore influence the effectiveness of the dip. Consequently wherever possible, and this was in the majority of cases, the sheep were dipped with 1-3 in. of wool as would be the case under ordinary farming conditions. In a few cases sheep with 6-8 in. of wool were dipped, and this fact has been taken into account in the interpretation of results. In the large scale trials sheep were dipped under ordinary farm conditions.

RESULTS

Derris:

The data relating to the effect of derris on lice are given in Table I. The observation of Morrison and McLeod that derris produces a 100 per cent. initial kill was confirmed but it was found that after many months lice sometimes suddenly reappear. In Trials 1, 4 and 5 the strength of the dip was the usual ½ lb. derris per 100 gal. In trials 2 and 3 the concentration of derris was increased to the order of 2 lb. 100 gal. in the hope that a stronger dip would prevent the reappearance of lice. The sheep of Trial 3 were housed indoors, as they were part of a different experiment and in the 12 months up to the time of removal no lice appeared. In Trial 2, however, the sheep were free of lice for nearly a year but they did reappear eventually.

Another series of trials was conducted to determine the rate of deterioration of derris. Derris, as a horticultural insecticide, is known to deteriorate on continued exposure to light and air. Similarly in the fleece its effect diminishes either due to mechanical loss of the derris particles or to destruction of rotenone. To obtain some idea of the rate of destruction of rotenone a sample of derris was spread thinly over calico sheets in a well lighted room so that any deterioration due to air and light would be accelerated. At intervals, samples were taken for determination of rotenone content by chemical analysis and of biological activity by their effect on keds. The results are recorded in Table II. All lambs and hoggets were unshorn. It will be observed that over a period of 5 months the rotenone content fell from 4.2 per cent. to 3.1 per cent. but the toxicity to keds still remained at a high level though its effect on lice was poor.

As a confirmation of the extreme toxicity of rotenone to keds, an extract of derris containing rotenone was tested under ordinary farm conditions, by dipping 260 crossbred ewes and 40 Downcross lambs. The dip contained the rotenone in emulsion form at a concentration of .01 per cent. The keds were completely eradicated. In a small

TABLE I. DERRIS

Trial No.	Date of Dipping.	Sheep.	Louse Population.	Wt 100 gal (lb.)	Per cent Rotenone in Derris	Louse Population. Months after Dipping 0	3	6	9	12	18
1	8/11/43	3 CW ewes	++	½	7		+(1)				
	,,	,,	++	,,	,,				+(1)		
	18/2/44	,,	+	,,	,,			+(1)			
	,,	,,	+	,,	,,			+(3)			
2	26/9/44	3 CW ewes	++	2	8.3						+(1)
	,,	,,	++	1-1/3	,,					+(2)	
	,,	,,	++	2-2/3	,,					+(1)	
	,,	2 CW ewes	+++	Control	Not dipped	+++	–	+	+++	+++	
3	5/4/45	20 CW ewes 20 FW ewes	++	2	5						
4	20/6/46	9 CW ewes	++	½	4		+				
5	20/6/46	9 CW lambs	++	½	4		+				

NOTE: (i) The number in brackets after the + sign, thus +(1), indicates the number of sheep in the group on which lice reappeared.
(ii) Sheep were examined monthly but results are tabulated tri-monthly

TABLE II. DERRIS, ½ LB. 100 GAL.

Date.	Sheep.	Derris Exposure. (Days)	Rotenone. (Per cent.)	Before Dipping.		3 Weeks and After.		4 Months and After.	
				Keds.	Lice.	Keds.	Lice.	Keds.	Lice.
6/11/45	28 hoggets	0	4.2	+ +	–	–	–	–	–
23/11/45	2 hoggets	17	4.2	+ +	–	–	–	–	–
14/1/46	3 lambs	69	3.9	+ +	–	–	–	–	–
20/6/46	3 lambs	155	3.1	+	+ +	–	+ (1)	–	+ (1)
20/6/46	3 lambs	0	4.2	+	+ +	–	–	–	+
20/6/46	3 lambs	155	3.1	+	+	–	+	–	+

scale trial using the same material the fleece remained toxic to keds for 4 months. The concentration of derris recommended by Morrison and McLeod and used in the field was ½ lb. per 100 gal. At 5 per cent. rotenone content this is equivalent to a concentration of rotenone of 0.0025 per cent. At this strength complete control of keds was achieved and the period of toxicity was 4-5 weeks. Trials were made to determine the minimum concentration required to control keds, with the following results:—

(i) 0.0016 per cent. rotenone (½ lb. of 3.3 per cent. derris per 100 gal.) complete control. Toxic at 3 weeks but broke down at 6 weeks.

(ii) 0.00066 per cent. rotenone (one-fifth lb. of 3.3 per cent. derris per 100 gal.) complete control but fleece was not 100 per cent. toxic to keds introduced 3 weeks after dipping.

(iii) 0.00025 per cent. rotenone (one-thirteenth lb. of 3.3 per cent. derris per 100 gal.) incomplete control. Some pupae hatched and survived. These confirm the original observations regarding the use of ½ lb. derris per 100 gal. and indicate that it would be unwise to recommend a lower concentration if a reasonable margin of safety is required.

Bentonite-Sulphur:

The composition of the American material was stated to be 70 per cent. bentonite and 30 per cent. sulphur and was prepared by fusing the sulphur with bentonite and then grinding to a fine powder after it had cooled. The finer details of the method of preparation were not available. The material used here was made as follows: 5 lb. of bentonite was thoroughly dried in an air oven and ground in a 12 in. ball mill for 3 hours, 2½ lb. of sulphur were added, the mixture ground for 8 hours, then heated to 160 C. for 1 hour, cooled and reground for 12 hours. A sieve analysis showed that 96 per cent. passed a 100 mesh and 87 per cent. passed a 200 mesh.

In 1940 trials were carried out with this material on 13 sheep heavily infested with keds. Dip concentrations were varied from 15 lb. to 60 lb. per 100 gal. In no case was a 100 per cent. initial kill produced and many keds survived the dipping. Similar results were obtained again in 1945 when 8 lambs were dipped in concentrations varying from 5-10 lb. per 100 gal.

Jet-pulverised bentonite-sulphur plus 2½ per cent. of sulphite lye was then tried. Some keds survived the dipping but within 4 months all were dead. On the other hand a similar dip a few months later failed to control either keds or lice. To test the period of protection 450 ewe lambs were dipped under practical farming conditions in a bentonite-sulphur (7 lb.) plus derris (½ lb.) mixture. The initial kill was 100 per cent. probably due to the derris. After 7 months' isolation the lambs became mixed with ked infested ewes. At mustering 10 months after dipping the lambs were lightly infested. By 1946 some of the American material was available but it contained rotenone. It was therefore difficult to separate the effects of the two components. At a concentration of 8 lb. per 100 gal. there was a 100 per cent. kill of keds but some lice survived though they too disappeared after 2 months.

The results with bentonite-sulphur have therefore been disappointing. Not only is a 100 per cent. initial kill not obtained but the claims of long protection have not been substantiated.

TABLE III DDT

Material	Date of Dipping	Sheep	D D T* Concn in Bath (Per cent)	Parasite Population		Ked Control Incomplete — Complete —	Louse Population				
				Keds	Lice		Months after Dipping				
							0	1	2	3	4
D D T-Talc	25 2 46	1 lamb	045	+ +							
,,	25 2 46	2 CW ewes	045		− −			—	—	—	
,,	16 7 46	2 CW ewes	03	−	− + +		—				
D D T-Sulphur	16 4 46	1 lamb	02								
,,	.	1 CW ewe	02				—	—	+		
Sulphur only	,	1 lamb		− +							
D D T-Derris	22 11 46	1 FW ewe	02	+ +			—			—	
,,	,,	1 FW ewe	02	− +	+ + +		—	—	—		
,,	,,	1 CW ewe	02		+ + +		—	—	+		
D D T Emulsion	22 11 46	1 FW ewe	1	+							
,,	,,	1 FW ewe	1	+	+			—	—		
,,	,,	1 CW ewe	1	—	+				−		
,,	26 2 47	3 FW wethers	01		− +	—		+ (2)			

D.D.T.:

The D.D.T. dip was prepared in a number of forms. All D.D.T. concentrations are quoted in terms of the pure compound.

(1) D.D.T.-talc. The D.D.T. was dissolved in light naptha and acetone, mixed with talc, the solvent evaporated off and the D.D.T.-talc pulverized. The powder was 20 per cent. pure D.D.T., 80 per cent. talc.

(2) D.D.T.-sulphur. This is a commercial preparation containing 20 per cent. D.D.T. and 80 per cent. wettable sulphur, jet pulverized. The D.D.T. in this case was 50 per cent. pure.

(3) D.D.T.-derris. Equal parts by weight of pure D.D.T. and derris powder were ground together thoroughly in a mill. The D.D.T. particles adhered to the derris and were dispersed in water with slightly greater difficulty than derris alone.

(4) D.D.T. emulsion. This was made according to the recommendation of Mitchell and Heath. Pure D.D.T. was dissolved in benzene and emulsified in water using sodium oleate and casein as emulsifying agents on 22/11/46 and sulphonated castor oil on 26/2/46. The concentrated emulsion (10 per cent. D.D.T.) was then diluted by pouring into the water of the dip. The emulsion was stable, with particle size about 2-3μ.

The results are set out in Table III. In the action on keds complete control was achieved in all cases except the .01 per cent. emulsion on 26/2/47. This was the lowest concentration reached and it seems therefore that the limiting concentration is of this order. The effect of D.D.T. on lice was not satisfactory, for while in all cases the initial kill was 100 per cent. the lice began to reappear after 2-3 months.

A small scale test on the period of persistence of toxicity of D.D.T. showed it to be 6 weeks in the case of D.D.T.-sulphur at a concentration of .02 per cent. and 3 months in the case of 0.1 per cent. D.D.T. emulsion.

Benzene hexachloride:

The benzene hexachloride used was the crude commercial preparation containing 12-13 per cent. of the γ-isomer. The concentrations used were of the order 0.00030 per cent.-0.0125 per cent. All concentrations throughout this work are expressed in terms of the γ-isomer. The benzene hexachloride dips were also prepared in a variety of forms:—

(1) Emulsions form. The dip used on 12/7/46 was a commercial dip in which the benzene hexachloride had been dissolved in phenol before emulsification. In that used on 22/11/46, the benzene hexachloride was dissolved in benzene and emulsified with sodium oleate and casein in the same way as the D.D.T. emulsion. The dip used on 26/2/47 was similar except that the emulsifying agent used was sulphonated castor oil. In these emulsions the majority of the particles were of the order of 2-3μ diameter, though some were considerably larger, up to 30μ. This is partly accounted for by the fact that a fraction of the benzene hexachloride is not soluble in benzene.

(2) Benzene-hexachloride—derris powder. As with D.D.T. an attempt was made to absorb the benzene hexachloride on the derris particles, relying on the latter to produce the suspension. The stickiness of the benzene hexachloride made the grinding together difficult. Nevertheless a suspension was made containing particles distributed throughout the whole range 1-30μ, and these sedimented rather more rapidly than derris alone. The dispersible benzene-hexachloride—derris mixture was somewhat better in physical properties.

(3) Dispersible powder. In this preparation, which was a commercial one, the benzene hexachloride had been absorbed on a solid dispersing agent, there being equal parts of benzene hexachloride and dispersing agent. This powder dispersed well. The particle size varied, most particles being in the range 1-8μ though some were as large as 15-20μ and these tended to settle out.

The results with these dips are set out in Table IV.

Except in the extremely dilute dips, complete control of keds was achieved. The dipping of 100 ewes and 100 lambs on 4/2/47 was carried out on a farm under practical farming conditions. The ewes were newly shorn and very lightly infested with keds but the lambs were unshorn and were all very heavily infested. The ewes were put through the dip first and the lambs last. They were examined 8 days later and again 67 days after dipping. No live keds were found at any time.

The period of toxicity of benzene hexachloride in the fleece was determined approximately by putting 20-30 live keds on to the sheep at various intervals after dipping and then counting the number of live and dead keds a week later. As soon as 1 ked survived it was considered that the fleece was no longer toxic. At 0.0030 per cent. (dispersible benzene hexachloride) the fleece remained toxic for 3 months; at 0.0050 per cent. (emulsion) it was toxic for 4 months and at 0.0125 per cent. it remained toxic for 5-6 months. In all these experiments concerning period of persistence of toxicity, the sheep used had 2-3 in. of wool at dipping.

The trials in the very dilute dips om 16/6/47 and 10/7/47 were designed to determine the minimum toxic concentration of benzene hexachloride. The results showed that at 0.0012 per cent. complete control was effected and that the fleece was toxic to introduced keds for a period up to 9 weeks. The 0.00060 per cent. dip also gave complete control but the fleece was not toxic to keds introduced three weeks after dipping. The 0.00030 per cent. dips, on the other hand, on both occasions failed to give complete control. A concentration of 0.0006 per cent. must therefore be regarded as the lowest limit.

The effect of benzene hexachloride on lice is less definite, because the sheep must be examined over a longer period of time. In all cases a 100 per cent. initial kill was achieved. At the time of writing lice have appeared on one sheep 5 months after dipping. In this case the dip was a derris-dispersible benzene hexachloride one which, along with the derris-benzene hexachloride mixture, was not entirely satisfactory in physical properties and was in any case at a low concentration—0.0015 per cent. In all other cases at benzene hexachloride concentrations varying from 0.003 per cent.-0.0125 per cent. no lice have yet appeared though the time since dipping varies from 6-12 months. No lice have yet appeared on the sheep dipped in the very dilute dips, 0.0003 per cent.-0.0012 per cent. though in these cases it is still only 2 months from dipping.

Table IX Benzene Hexachloride

Material	Date of Dipping	Sheep	γ isomer (Benzene hexachloride Concn in Bath) (Per cent)	Parasite Population		Ked Control Incomplete Complete	Louse Population				
							Months after Dipping				
				Keds	Lice		0	3	6	9	12
Benzene hexachloride Emulsion	12 7 46	5 lambs	0050								
	22 11 46	3 FW ewes	0125								
	26 2 47	3 FW ewes	0030		+						
Benzene hexachloride-Derris	22 11 46	3 FW ewes	0030		- - -						
Dispersible Benzene hexachloride and Derris	22 11 46	3 FW ewes	0015		– –				– (1)		
Dispersible Benzene hexachloride Powder	22 11 46	3 FW ewes	(125		+						
	,	3 FW ewes	0030								
	4 2 47	104 FW ewes 102 lambs	0030								
	26 2 47	3 FW wethers	0030		– +						
	16 6 47	2 C W ewes	0012								
	..	2 C W ewes	00060								
	,	2 C W ewes	00030								
	10 7 47	2 C W hoggets	00030								

DISCUSSION

With regard to derris, the present work together with field data over several years has served to confirm the extreme toxicity of either derris powder or of rotenone to keds. Not only do they destroy all living keds at dipping but they remain in the fleece for a sufficient time to kill all young keds emerging from pupae. Provided the flock is not subjected to reinfestation with keds from undipped sheep, one dipping suffices to eradicate keds entirely. It follows that many of the materials at present incorporated in compounded dips are superfluous. A further point in favour of derris is that it is non-poisonous and is much cheaper than any other known dip.

On the other hand it must be admitted that derris does not give the same control over lice. In all cases derris kills all adult lice. The results show that the sheep remain apparently free of lice for several months and that in some cases they then suddenly reappear. Why they should not appear, often until 9-10 months after dipping, is not known. Since the examination of the sheep is very thorough, it can scarcely be due to the louse eggs hatching within a few weeks of dipping or the young lice would have been observed earlier. No explanation of this can be given. With most lice such as human lice and pig lice, the period of incubation is only a few weeks. In this respect very little is known of the life history of the sheep louse, so it is difficult to discuss what factors cause the reappearance of lice after such long periods. What is known, from experiments at the College, is that keds and particularly lice on isolated sheep vary in number from time to time with the nutrition and thrift of the host, and with seasonal conditions. In one case, two lice-infested sheep in low condition were found to be free of lice after shearing and an improvement in condition was noted. This, however cannot alter the fact that derris does not completely control lice.

The other disadvantage of derris, namely that it sometimes causes lameness, is also unexplained. The lameness is of a temporary nature appearing within a few days after dipping and rarely lasts for more than 2-3 weeks (2). No mortality occurs but it gives the sheep a setback. Outbreaks occur sporadically, affect some flocks and not others, and affect varying numbers within a flock. The only clue known at present is that it seldom if ever occurs in sheep dipped on the same day as the dip is made up, but occurs more frequently on subsequent days, or after large numbers have been put through the dip. As far as is known, derris extracts containing rotenone do not cause lameness.

The future of derris powder as a dip remains uncertain so long as the lameness problem is unsolved. Even with lameness and incomplete control of lice, derris still has a place until the ideal dip is discovered. For instance, the number of flocks infested with lice is small, and derris is so cheap and effective against keds that its use will probably continue. Where it is easy to empty and refill the dip in order to use a fresh derris dip each day, derris is safe and reliable. For dipping ewes with lambs it is certainly safer than the standard arsenic and phenolic dips.

The results with bentonite-sulphur were disappointing in view of the claims made for it. This can hardly be due to the method of preparation, and the fact that recently imported material contains rotenone leads one to suspect that the American workers have subsequently been unable to substantiate their original claims.

D.D.T. gave effective control of keds, thus confirming the observations of Heath (4). The concentration of D.D.T. might possibly be reduced below Heath's lowest concentration of 0.125 per cent. since our results show control of keds down to a D.D.T. concentration 0.02 per cent. Some greater margin of safety may, however, be necessary, for at 0.01 per cent. ked control was not achieved. Unfortunately its efficiency in lice control is not good, in fact it is less effective than derris. Though a 100 per cent. initial kill was invariably achieved, lice reappeared within 2-3 months. Therefore like derris, it cannot be considered as a general purpose dip for both keds and lice but could be used for flocks infested with keds only. As far as is known D.D.T. is harmless to sheep but insufficient field data is available. Only large scale practical use can prove whether or not it will exhibit any harmful effects. If it does not there may be a place for it as a non-toxic, safe dip for ked infested flocks.

Benzene hexachloride has given very promising results. Complete control of keds was achieved at all concentrations down to 0.0006 per cent. It would be advisable to use a considerably stronger dip than this however, to give a margin of safety, to take account of the stripping out of the benzene hexachloride as the sheep are dipped, and to confer on the sheep some period of freedom from reinfestation. Until more experience is gained a concentration of the order of 0.003 can safely be recommended and will ensure complete control at one dipping together with 3-4 months freedom from reinfestation. A dip at such strengths should be capable of manufacture at a relatively low cost.

The effect of benzene hexachloride on lice is certainly better than that of any of the other materials tested. The initial kill was in all cases 100 per cent. With one exception no lice have reappeared though some of the sheep have been dipped for up to 12 months. The sole exception was in one of three ewes dipped in a derris—benzene-hexachloride mixture at a concentration of 0.0015 per cent. where live lice have been found. It appears therefore that benzene hexachloride is also very effective against lice. This, nevertheless, must be accepted with some caution yet, for experience with derris has shown that there is still time for lice to reappear. In addition, large scale trials against lice have not been possible owing to the difficulty of locating lousy flocks. If the effectiveness of control of lice is confirmed, benzene hexachloride promises to provide a single component dip which will control both keds and lice. Our experiments do not differentiate between emulsion or suspension forms and it is probable that both have roughly equal potentialities.

During last autumn several flocks have been dipped in a commercial dip containing benzene hexachloride and phenol. It was with some astonishment that reports were received of lameness with this dip. McLean (2) has investigated these and has found that the condition is apparently identical with the derris lameness but the outbreaks are less frequent and not so widely distributed. Thus, whilst benzene hexachloride shows every promise of being an outstanding material as a sheep dip there is still this problem to be overcome before its use can be advocated with complete confidence.

There is one further point about both D.D.T. and benzene hexachloride which is brought out by Australian workers (private communication N.P.H., Graham) but which has not been studied here. That is the 'stripping out' effect, whereby the D.D.T. or benzene hexachloride is taken out of the dip by absorption on the wool as the sheep are dipped. This results in the concentration in the dip falling as more sheep are put through. The optimum particle size in the dip, the rate of topping-up with more D.D.T. or benzene hexachloride have still to be decided.

ACKNOWLEDGMENTS

Our thanks are due to the Department of Scientific and Industrial Research for financial assistance in the carrying out of this work, to V. R. Clark for assistance in the care and examination of the sheep, to A. F. R. Adams for estimation of rotenone content of derris samples, and to L. Morrison for assistance in the early trials.

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ADDENDUM

After twelve months had elapsed since the benzene hexachloride trials at strengths of 0·003 per cent. and greater (Table IV) against lice no lice had appeared confirming that at these strengths the dip is effective. At the very weak concentrations there were still no lice after 8 months at dipping strength of 0·0012 per cent., but lice had appeared and multiplied after 4 months on those dipped at 0·0006 per cent A flock of 350 heavily louse-infested sheep had been dipped under practical conditions at a strength of 0·003 per cent. and no lice had reappeared after 6 months

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CLASSIFICATION OF BARLEY VARIETIES IN NEW ZEALAND

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(Received for publication 31 January 1949)

Summary

Various morphological characters of barley have been reviewed for use in the classification of varieties grown in New Zealand. Characters most stable under varying environments were utilized for individual variety descriptions and for the arrangement of identification keys.

INTRODUCTION

THE release by plant breeders of numerous agricultural varieties which are closely similar from the taxonomic viewpoint has resulted in difficulty and often confusion in their identification. Varietal performance in barley is commonly measured by physiological characters such as yield, standing ability, disease resistance, and malting quality. However, unless these physiological characters are associated with distinctive morphological characters, absolute identification under diverse environmental conditions is extremely difficult, if not impossible. A further complication arises from the fact that several of the newer agricultural varieties have been derived by mass selection. Individual plants within an agricultural variety may thus show considerable variation.

As long as morphological characters themselves do not have an important effect on the physiological characteristics that determine the value of the variety, they are of minor importance to the plant breeder and of no economic importance to the farmer. However, morphological differences are used by barley specialists in the recognition of agricultural varieties. After acquiring an accurate knowledge of varieties, the presence of 'rogues' or 'off-types' in crops is readily detected. A pure line of grain with high even germination is a necessity for the malting process and also to ensure high yields with even maturity. This last factor is essential for good condition of harvested grain and for the even behaviour of grain on the malting floor.

PREVIOUS CLASSIFICATIONS

The development of systems of barley classification may be traced from 1753 when Linnaeus in his 'Species Plantarum' first delimited species. Linnaeus and his followers utilized spike characters alone for classification.

Field studies were attempted by Kornicke in 1885, but as these were confined to one climatic region and a small range of material, the importance of stability and variability of characters was not apparent.

The study of stability in the field under different environmental conditions was commenced by Harlan (1914, 1918) who realized the need for studying the material in the field and following its development from seeding to maturity under different regional and climatic conditions.

These principles advocated by Harlan, followed by Bell (1937), and later by Aberg and Wiebe (1946) have been followed in this study.

The species classification adopted here to distinguish the small number of agricultural varieties in New Zealand follows that of Kornicke with minor amendments by Beaven (1947).

Hordeum sativum Jess (H. vulgare Kcke.)

Sub-species or Races (Fig. 1)

1. Spike of six rows of spikelets, all fertile.
   A. Wide with short internodes, *H. hexastichum L.*
   B. Narrow, with long internodes, *H. vulgare L. (H. hexastichum Kcke.)*
2. Spike of six rows of spikelets, all fertile—two median rows normal, four lateral rows diminutive and without awns. *H. intermedium Kcke.*
3. Spike with two median rows of spikelets fertile and four lateral rows infertile or staminate.
   A. Wide, with short internodes, *H. zeocriton L.*
   B. Narrow, with long internodes, *H. distichum L.*
4. Spike with two median rows of spikelets fertile and four lateral rows rudimentary and without floral organs. *H. decipiens Steudel.*

METHOD

The classification studies were carried out at Lincoln between 1945 and 1948 with both autumn- and spring-sown material. Confirmatory observations were made by officers of the Department of Agriculture and by the writer throughout the principal barley districts of Marlborough, Canterbury, and Otago.

The varieties at Lincoln were sown at intervals of four inches and two inches and also at normal seeding rate, in rows one foot apart. Detailed notes were recorded in the juvenile stage, shooting period, flowering period, at spike emergence, two weeks after spike emergence, and at binder and header ripe stages. A sample of fifty mature plants was pulled and measurements were recorded in the laboratory. From this sample ten spikes were selected for detailed study.

Varieties described include those grown commercially and those showing promise in trials with possibilities of release. With the exception of Gisborne, all the varieties grown in the Dominion are of overseas origin. The present observations have, therefore, been compared with relevant descriptions in overseas literature. Descriptions of particular varieties and the sources of information include the following:—Gisborne (Archer 1922); Kenia (Anon. 1928); Cape and Pryor (Vears 1935); Chevallier, Plumage Archer, Spratt Archer, and Victory (Bell 1937); Wong (Love and Craig 1943); Pioneer (Bell 1944); Prefect (Anon. 1944); Newal (Aberg and Wiebe 1946); Golden Archer (Beaven 1947).

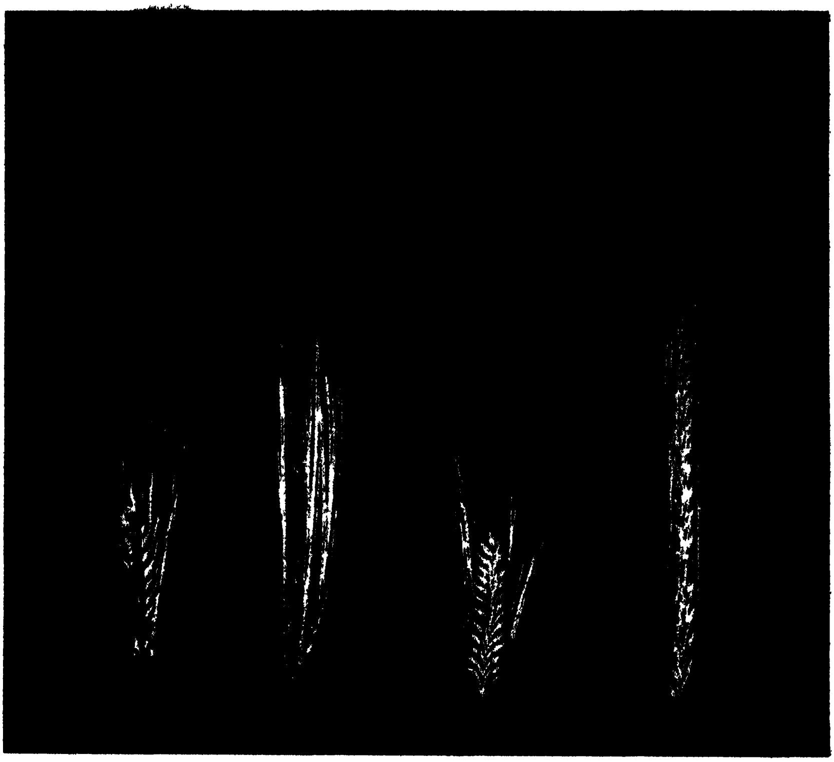

FIG. 1.—Sub-species of Hordeum sativum, Jess. Left to right: *H. hexastichum* L.; *H. vulgare* L.; *H. zeocriton* L.; *H. distichum* L.

THE BARLEY PLANT (Fig. 2)

Stem: The cylindrical stem consists of hollow internodes separated by solid nodes at which the leaves arise. The stem and the spike are joined by the collar. Environment and variety determine the length of the stem and the number of stems per plant.

Leaf: (Fig. 3). A single leaf arises at each node and these are borne alternately on either side of the stem. The leaf consists of a sheath, ligule, auricle, and blade. The sheath encloses the stem and is split to the base on the side opposite the blade. In most varieties the sheath is glabrous, but in some it is covered with hairs. The ligule extends upward at the union of the blade and sheath. At its base the blade extends into two claw-shaped auricles which clasp the stem. The uppermost leaf which is called the flagleaf is normally much smaller than the others, and in some varieties is curled or rolled. The blade surface is rough and covered usually with a waxy deposit.

Spike: The spike is made up of flowers attached at nodes of the rachis. The rachis is solid and consists of alternate flattened nodes and internodes. The groups of three spikelets are attached on alternate sides of the rachis, one group above the other. In two-rowed varieties only the central spikelet of each group is fertile and produces a kernel.

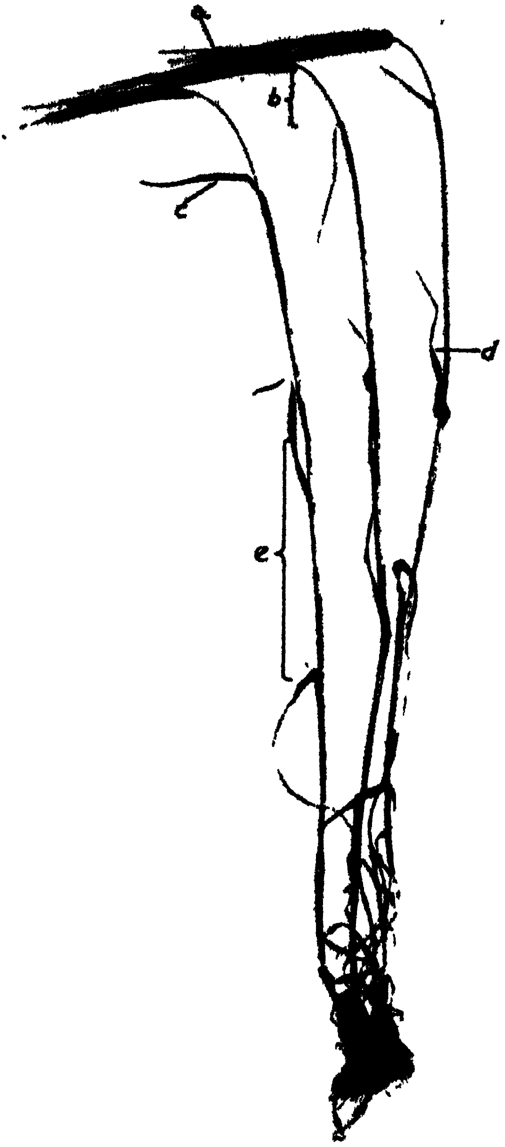

FIG. 2—Barley plant (a) Spike, (b) Unsheathed portion of peduncle, (c) Flagleaf; (d) Leaf Blade, (e) Internode

Spikelet (Fig. 4): A spikelet consists of two glumes and the floret. The two outer glumes are linear, flat, possess small nerves, and terminate in an awn.

Floret (Fig. 5): The floret consists of the lemma, palea, rachilla, and the male and female flower parts. The lemma has five nerves and terminates in a straight awn. The lateral and marginal nerves have, in

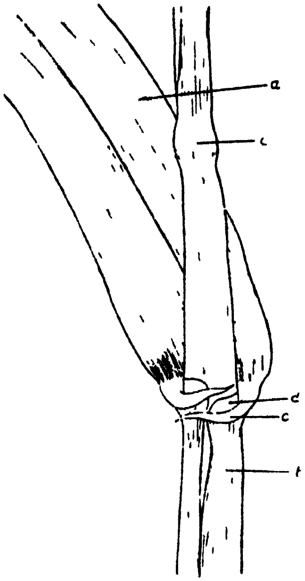

FIG. 3.—Junction of blade and leaf sheath: (a) Blade; (b) Leaf Sheath, (c) Auricle; (d) Ligule; (e) Node. (After Aberg and Wiebe.)

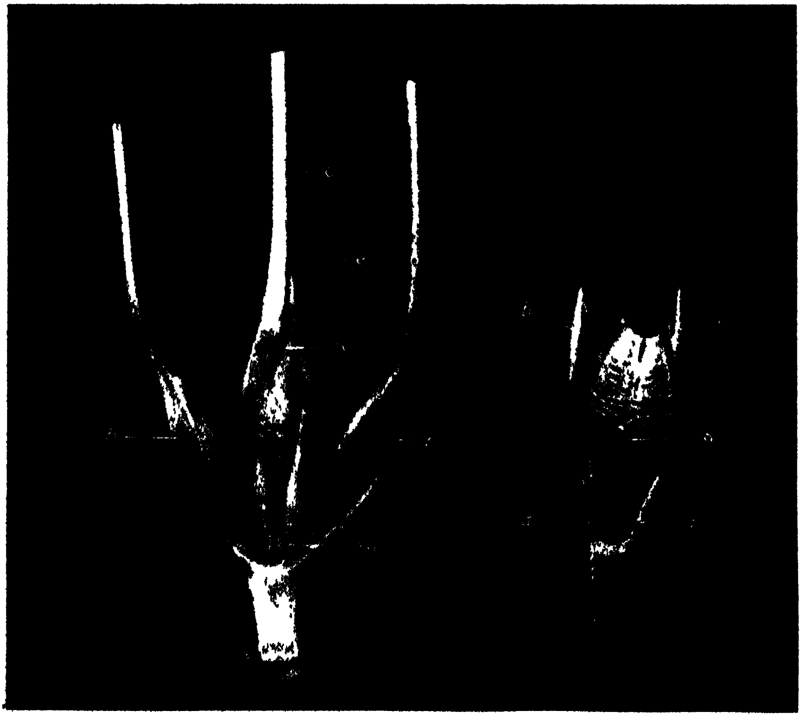

FIG. 4.—Spikelet groups: Left, Six-rowed barley; Right, Two-rowed barley; (a) central kernel; (b) lateral kernels (six-rowed) or sterile lateral florets (two-rowed); (c) awn; (d) outer glumes; (e) glume awns.

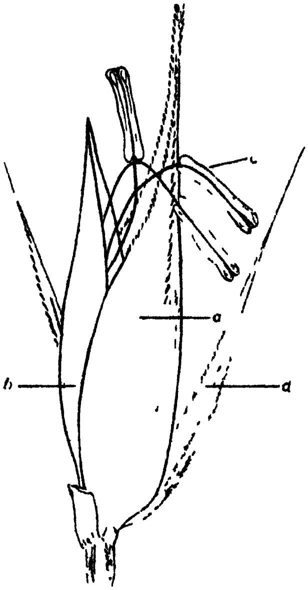

FIG. 5.—Barley floret. (a) lemma; (b) palea, (c) anther; (d) outer glume (After Aberg and Wiebe)

some cases, numerous small teeth, but are usually smooth. The lemma may have either a depression or a transverse crease at its base on the dorsal side just above the point of attachment. The awn may be rough or smooth. The palea is curved inwards between its two nerves and is turned inwards at the margins. The rachilla is a continuation of the axis of the spikelet and lies within the crease of the kernel. It is covered with either long or short hairs.

Kernel: The barley kernel consists of the caryopsis and the lemma, palea and rachilla. In most varieties the lemma and palea adhere to the caryopsis, whereas in others they are free and the caryopsis threshes out like wheat.

DESCRIPTIVE CHARACTERS

I. GROWTH CHARACTERS

This section deals with the manner in which the plant grows and its relation to environment and disease.

Spring or Winter Growth Habit: Although it is possible either to autumn- or spring-sow a spring variety and obtain a mature crop, it is found that there is incomplete development of a true winter variety when spring-sown. The only winter varieties in New Zealand are Pioneer and Wong, which are, as yet, not commercially grown. Prefect is described overseas (Anon., 1944) as a winter variety because of a high degree of winter hardiness and its prostrate juvenile habit. However, Prefect completes development and produces a crop when spring-sown. Winter varieties are characterized by a flat, prostrate, juvenile growth habit, while spring varieties tend to be more erect.

Juvenile Habit: In the juvenile leafy stage of growth the barley plant may be 'erect,' 'semi-erect,' or 'prostrate,' depending on the angle of the tillers with the soil (Fig. 6). The density of the strike somewhat modifies the juvenile habit—spaced plants invariably tend to be more prostrate than crowded ones.

Spike Emergence: Emergence is a more accurate index of rate of development than is time of ripening. Three groups are distinguished 'early,' 'midseason,' and 'late,' with a difference in time of emergence of from five to ten days between groups.

Disease Reaction: The question of varietal susceptibility or resistance to disease is of vital economic importance. In New Zealand the most serious diseases have been controlled by various seed treatments. Pickling with fungicides and, latterly, dusting with organic mercurial dusts have effectively controlled covered smut *(Ustilago hordei).* In 1925 hot-water treatment was first used to control loose smut *(Ustilago nuda)* and leaf stripe *(Helminthosporium gramineum);* today it is only on rare occasions that smut can be found in malting barley crops. Rust (*Puccinia* spp.) is prevalent in certain seasons causing shrivelled grain and reduced yield. Powdery mildew *(Erysiphe graminis hordei)* may usually be found in the early part of the season.

Wong is resistant to mildew, as reported by Love and Craig (1943), and Black Skinless shows some degree of resistance. Shands and Arny (1944) report the Newal is moderately resistant to stripe. However, definite reactions cannot be recorded until possible physiologic races of diseases present in the Dominion are determined by artificial innoculations

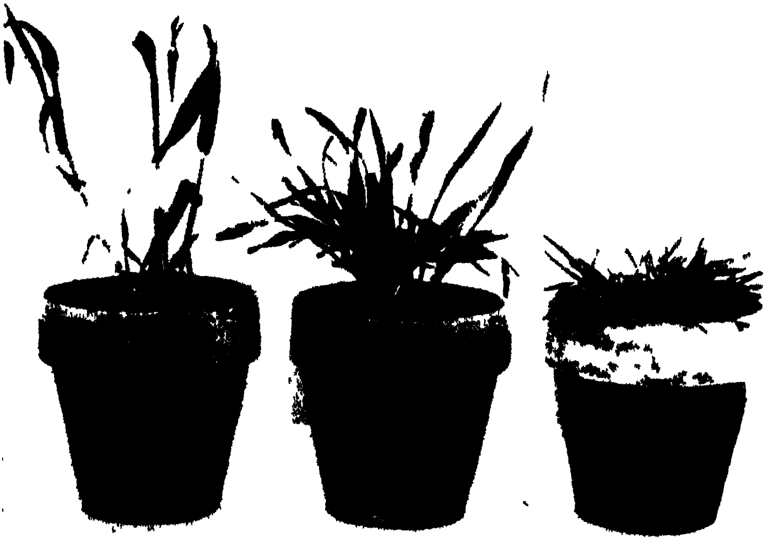

FIG. 6.—Juvenile growth habit: Left to right, erect, semi-erect, prostrate.

II. LEAF CHARACTERS

Hairiness of the Leaf Sheath: The lower leaf sheaths of some varieties are covered with hairs which drop off or are broken off as the plant matures. Most varieties in this country are without hairs on the sheath.

Pigmentation of Sheaths and Auricles: The red or purple coloration found in the leaf sheaths at the base of the plant and frequently on the auricles of some varieties is subject to seasonal and environmental fluctuations. The intensity of the colour is more pronounced after periods of prolonged cold weather. As a rule the two-row varieties develop some degree of pigmentation at the base of the culms and often on the auricles, while six-row varieties are always free from pigment.

Colour and Size of Leaves: Length, width, and colour of leaves show considerable variation, but because of difficulty in description and the effect of environment, are used only where differences are outstanding.

Size and Habit of Flagleaf: The size and habit of the flagleaf are observed and recorded at the time of ear emergence. Because of their variability they are included in the variety descriptions as subsidiary descriptive characters.

III. STEM CHARACTERS

Height of Plant: To determine the height of a plant, the distance from the ground to the base of the spike is measured. This character is easily influenced by environment, being useful only when two varieties differ significantly in height under all conditions. The groups used are 'tall,' 'midtall' and 'short.'

Length of Unsheathed Peduncle: The peduncle is that portion of the stem from the last node to the base of the spike. It may be completely sheathed or have a portion unsheathed below the base of the spike. Under adverse growing conditions the peduncle of a variety may be completely sheathed although not normally so. This character offers some assistance in the identification of two-row, lax-eared types.

Strength of Straw: One of the important objectives in barley selection has been the improvement of straw strength. Certain six-row, feed varieties and some of the older two-row varieties in New Zealand are weak in this respect. Straw strength is affected by a number of factors, including diameter and quality of the straw, height of plant, nature of the root system, the stage of plant growth in relation to bad weather, the height and position of the spike and the incidence of stem diseases. Varieties are classed as 'strong,' 'medium strong,' and 'weak.'

IV. SPIKE CHARACTERS

Real differences in spike characters are not shown until at least a fortnight after spike emergence. The most useful spike characters at this and later stages are the number of rows of fertile kernels, the length of awn, the barbing of the awn, the shape, length, and density of the spike, the attitude of the spike, the colour in the awns and glume awns and the deciduousness of the awns.

Fertility: In six-row barleys all three spikelets of the group at a node are fertile, giving six rows of grain around the rachis. In two-row barleys only the centre spikelet of each group of three at a node is fertile, resulting in two rows of grain around the rachis.

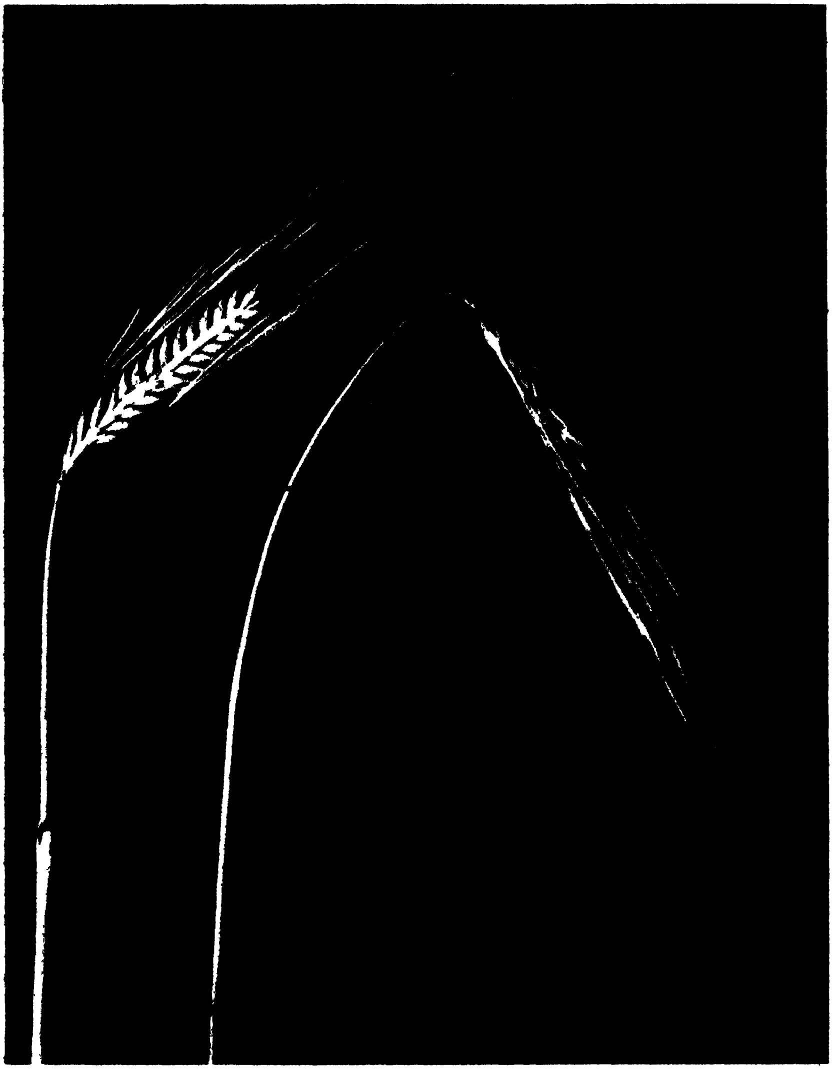

FIG. 7.—Spike shape and attitude: Left, dense and erect; right, lax and drooping.

Length of Awn: All varieties except one in this classification have long awns, about twice the length of the spike. Wong has short awns on the central rows of kernels and very short awns on the lateral rows.

Barbing of the Awn: Most varieties possess awns barbed along the entire length. The one exception in New Zealand is Newal, which has smooth awns.

Deciduousness of the Awns: This is an obvious field character, but is influenced by environment. Varietal differences also exist, and the variation in expression has been stated to be partly due to the ability of the plants to accumulate different quantities of ash in the awns (Pope 1945).

Spike Shape: Two groups of varieties can be distinguished: (1) the parallel type, where the sides are parallel throughout the length of the spike; and (2) the pyramidal type, where the sides converge towards the tip and sometimes towards the base of the spike.

Length of Spike: The existence of large fluctuations limits the use of the character but in some varieties the spikes are very long and in others very short. Three broad groups have been distinguished for descriptions: 'long', 'medium long', and 'short'.

Spike Density: In dense-eared varieties there are more spikelet groups per unit length of rachis than lax-eared varieties. Because of environmental fluctuations, density measurements are not stable within a variety and can therefore be used only where marked differences occur between varieties.

Spike Attitude: Two or three weeks after emergence the spike assumes various attitudes due to the curving of the peduncle or of the rachis at the base of the spike (Fig. 7). Lax-eared varieties assume a nodding habit, but the speed at which this is attained varies. In dense-eared barleys the spike is usually erect, but may finally bend over when the grain is ripe.

Pigmentation of the Spike: Before the grain begins to ripen there is a period when pigment on the lemma awns and glume awns and sometimes the lemma nerves and sterile lateral florets (of two-row varieties) reaches its maximum development. The six-row barleys and the Australian two-row varieties, Pryor and Research, do not develop pigment on

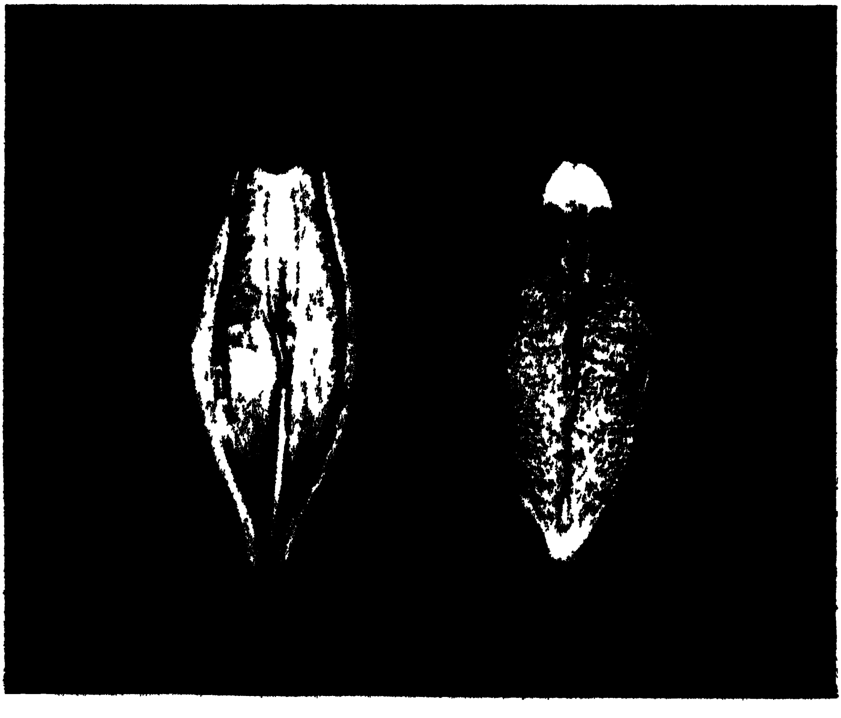

FIG 8—Kernel type: Left, covered; Right, naked.

the spikes. Black Skinless is an exception because the lemma and palea become deeply pigmented while the kernel is filling, but the colour fades as the grain ripens, leaving the kernel alone a deep purple colour. The intensity of pigmentation is influenced by weather conditions.

V. GRAIN CHARACTERS

Covered or Skinless Grain: In all varieties in New Zealand, except Black Skinless, the lemma and palea adhere to the caryopsis and are not removed in the threshing process (Fig. 8). Such barleys are termed 'covered' barleys in contrast with 'skinless' barleys.

Shape of Kernels: Samples of six-row barley can be recognized by the uneven size of the individual grains and by the twisted shape of some of the grains. Two-thirds of the grains in the sample will be twisted and somewhat smaller than the remainder. The smaller twisted grains are the lateral grains of the spike. The grain of six-row barleys is usually narrower than that of two-row barleys, which have even-sized symmetrical grains (Fig. 9).

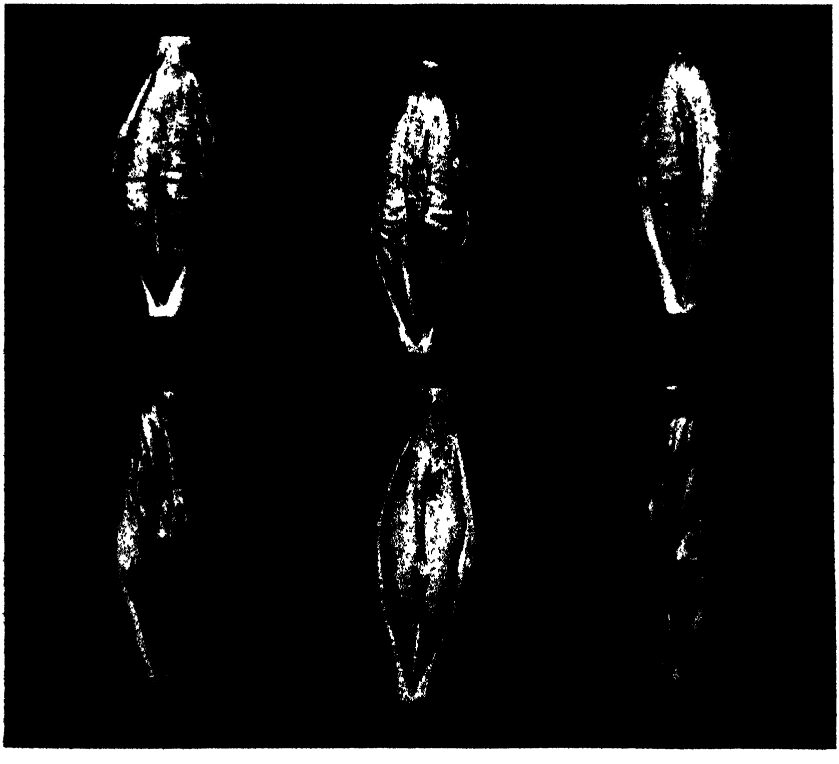

FIG. 9.—Kernel shape: Top row, even-sized symmetrical kernels of two-rowed barley; bottom row, symmetrical central kernel and smaller twisted lateral kernels of six-rowed barley.

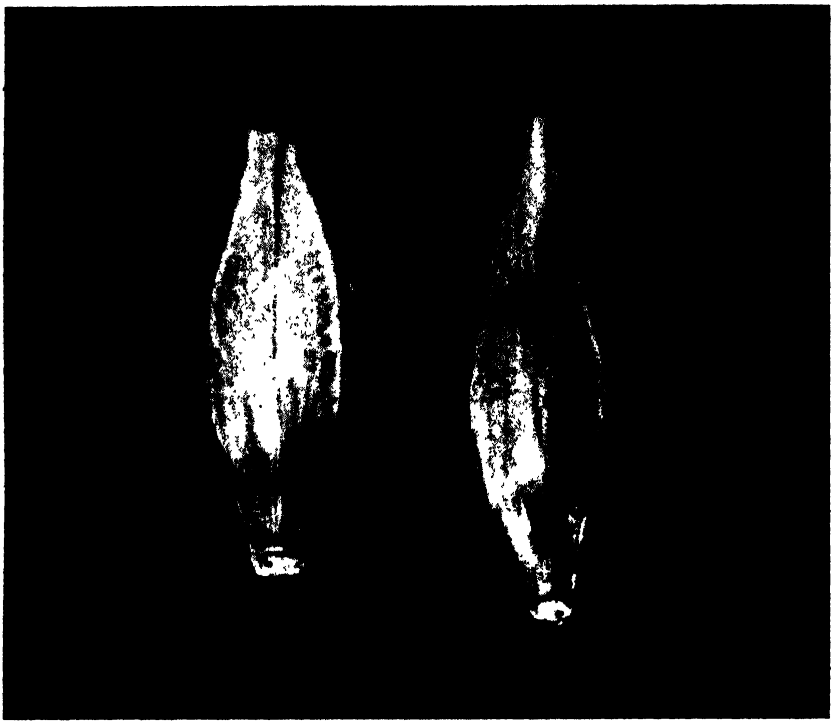

FIG. 10.—Base of the Grain: Left, transverse crease; Right, depression.

Length of Kernels: The only useful measurement of kernel size is length, because width and thickness vary too much with environmental conditions. Three groups are used: 'long', 'medium long', and 'short'.

Colour of Caryopsis: The following caryopsis colours are found in local barleys: blue, white, and purple-black. Hector (1936) states that variation in colour is due to the relative distribution of two pigments: a melanin compound which is black, and an anthocynin which is red in acid and blue in alkaline conditions. Harlan (1914) describes the interaction of these pigments as follows: 'White denotes the absence of all pigments; anthocyanin in the aleurone (alkaline) results in blue barley; a skinless barley with blue aleurone and red pericarp (anthocyanin in acid conditions) results in a purple barley.'

Base of the Grain: There may be either a depression or a transverse crease at the base of the lemma on the dorsal side (Fig. 10). The character is often not fully expressed in an individual grain, but it is stable within the group providing suitable grains are selected for examination.

Lemma Teeth: The lemma possesses five nerves, namely, the midnerve, the two lateral nerves next to the midnerve, and and two marginal nerves near the edge of the lemma. The midnerve is always without teeth, but the two lateral nerves may or may not possess teeth near the apex of the lemma (Fig. 11).

Rachilla Hairs: The rachilla may be covered with long straight hairs, or with short downy hairs giving a fuzzy appearance (Fig. 12). No significant differences in the length of the rachilla were found within the range of material tested.

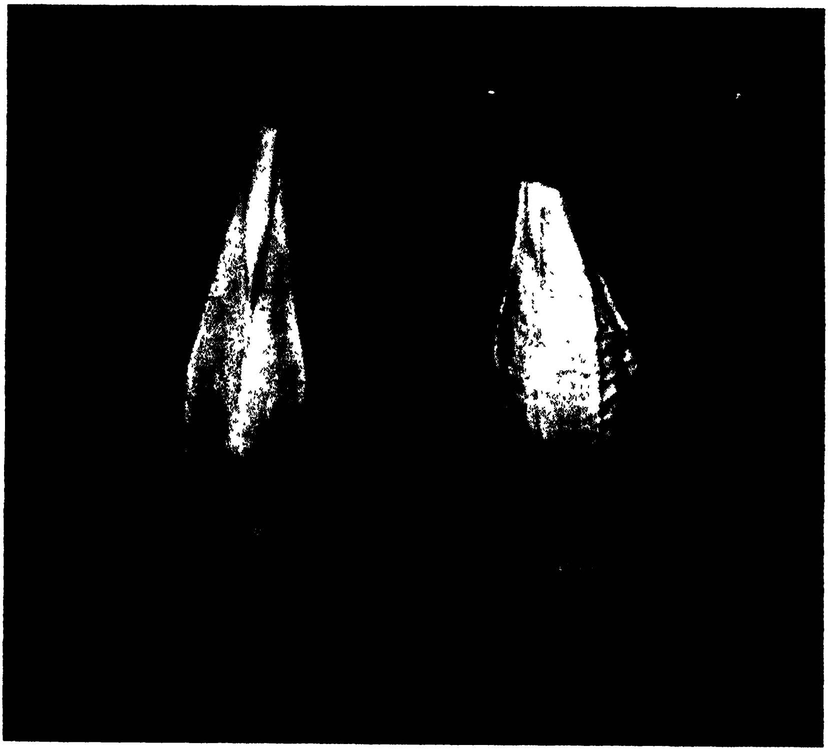

FIG. 11.—Lemma teeth: Left, teeth on lateral nerves; Right, no teeth on lateral nerves.

ORIGIN AND DESCRIPTION OF VARIETIES

The first group contains the two-row barleys arranged in alphabetical order, and the second group the six-row varieties.

TWO-ROW VARIETIES

CHEVALLIER

This very old variety was selected in England in 1820 and repeated selections have been made since then. One selection, Kinver Chevallier, has been the predominant variety for many years in most barley districts of New Zealand, providing good yields of attractive malting grain. Chevallier is now out of cultivation in England because of its long, weak straw. Lodging is frequent on heavy land in this country.

Description: Two-rowed spring barley; *Hordeum distichum* L.; juvenile growth habit semi-erect; plant late, tall; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf medium long, medium wide, rolled, twisted; top of peduncle mostly unsheathed; spike lax, long, parallel, nodding; awn long, barbed, pigmented; lemma with depression at base, no teeth on lateral nerves; rachilla short haired; kernels white, long, uniform shape and size (Fig. 13).

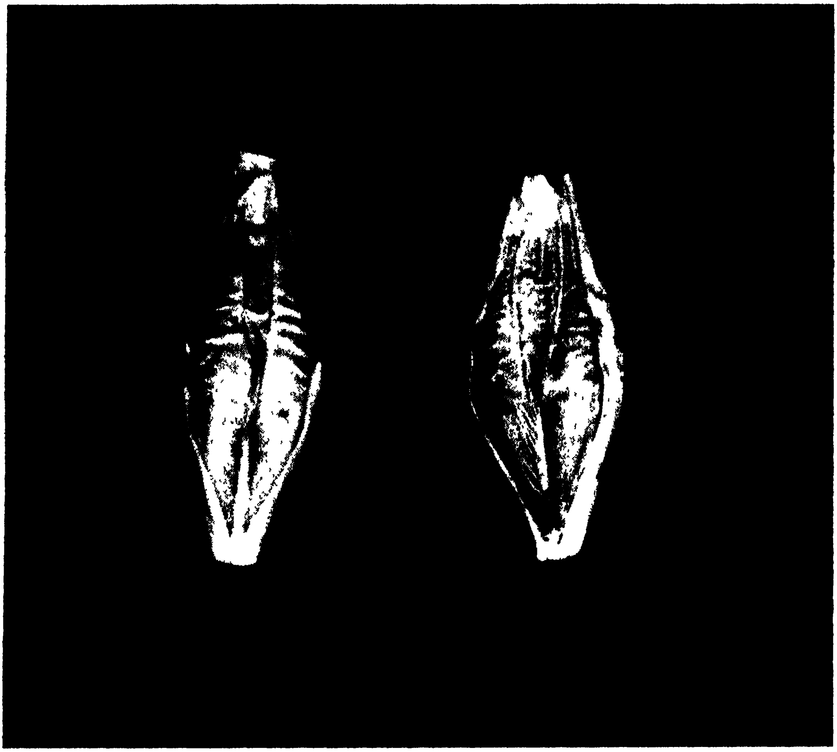

FIG. 12.—Rachilla hairs: Left, short hairs; right, long hairs.

GISBORNE

Gisborne was at one time popular in certain districts with the synonym of 'Wind-resistant', but did not prove superior to other varieties in this respect. Its origin is uncertain, but it is probably a New Zealand selection of Spratt. There is considerable variation in many characters within the line. The straw is moderately strong, and frequently the very dense spike assumes a twisted shape.

Description: Two-rowed spring barley, *Hordeum zeocriton* L., juvenile growth habit semi-erect; plant late, midtall; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf medium long, medium wide, not rolled, twisted; top of peduncle mostly unsheathed; spike dense, short, tapering, erect; awn long, barbed, pigmented; lemma with depression at base, no teeth on lateral nerves; rachilla short haired; kernels white, short, uniform shape and size (Fig. 14).

GOLDEN ARCHER

This is a mass selection from a cross between Plumage Archer and Spratt Archer made in England. In appearance it is very similar to Spratt Archer. In extensive field trials since being introduced in 1937, it has shown distinct promise as a high yielding, good quality barley with the ability to stand well until ready for harvesting. On the heaviest land Golden Archer has lodged to a slight extent. A substantial area of the variety was sown in Canterbury during the 1947-48 season.

Description: Two-rowed spring barley; *H. distichum* L.; juvenile growth habit semi-erect; plant late, short; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf short, narrow, rolled, twisted; top of peduncle mostly sheathed; spike lax, long, parallel, nodding; awn long, barbed, pigmented; lemma with depression at base, no teeth on lateral nerves; rachilla long haired; kernels white, medium long, uniform shape and size (Similar to Fig. 19).

GOLDTHORPE SPRATT

This variety was first grown in New Zealand in 1924, but has never been grown on an extensive scale. It has been popular on the lighter soils of Central Otago and Marlborough where it yields well. It is recognized as the best variety for malting, but is subject to lodging and shaking.

Description: Two-rowed spring barley; *H. zeocriton* L.; juvenile growth habit semi-erect; plant late, tall; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf medium long, narrow, rolled, twisted; top of peduncle mostly unsheathed; spike dense, medium long, parallel, erect to inclined; awn long, barbed, pigmented, deciduous; lemma with transverse crease at base, no teeth on lateral nerves; rachilla long haired; kernels white, long, uniform shape and size (Fig 15).

H.H. 12 CULTURE 9

Received in 1945 from the Plant Breeding Institute, Cambridge University, Culture 9 is a promising new unnamed selection from a cross between Maja and Spratt. It has a short, stiff straw, is early, gives a very good yield, but is only of average malting quality. Its best feature is its ability to stand until header-ripe without shaking or neck-break.

Description: Two-rowed spring barley; *H. distichum* L.; juvenile growth habit semi-erect; plant midseason, short, basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf medium long, narrow, rolled, twisted; top of peduncle invariably sheathed; spike lax; medium long, parallel, nodding; awn long, barbed, pigmented; lemma with depression at base, no teeth on lateral nerves; rachilla long haired; kernels white, long, uniform shape and size (similar to Fig. 16)

KENIA

Kenia is a Danish barley derived from the cross between Binder and Gold. In most respects it is similar to Culture 9.

Description Two-rowed spring barley; *H. distichum* L.; juvenile growth habit semi-erect; plant midseason, short; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf short, narrow, rolled, twisted; top of peduncle nearly always sheathed; spike lax; short, parallel, nodding; awn long, barbed, pigmented; lemma with depression at base, teeth on lateral nerves; rachilla long haired; kernels white, medium long, uniform shape and size (Fig. 16).

PIONEER

This is a recently developed winter malting barley from the Plant Breeding Institute, Cambridge University. It is a hybrid from a cross between Spratt Archer and Tschermak's two-row winter barley. Because of its winter hardiness, high yield, and good malting quality the variety may suit certain districts of New Zealand where autumn sowing is practised. Pioneer is definitely unsuitable for spring sowing.

Description: Two-rowed winter barley; *H. distichum* L.; juvenile growth habit prostrate; plant late, midtall; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf medium long, medium wide, rolled, not twisted; top of peduncle usually unsheathed; spike lax, long, parallel, nodding; awn long, barbed, pigmented; lemma with depression at base, no teeth on lateral nerves; rachilla long haired; kernels white, medium long, uniform shape and size (similar to Fig. 19).

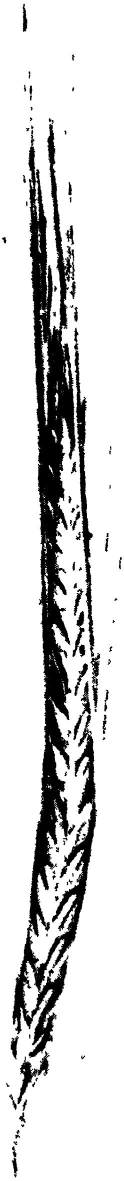

FIG. 13.—Chevallier.

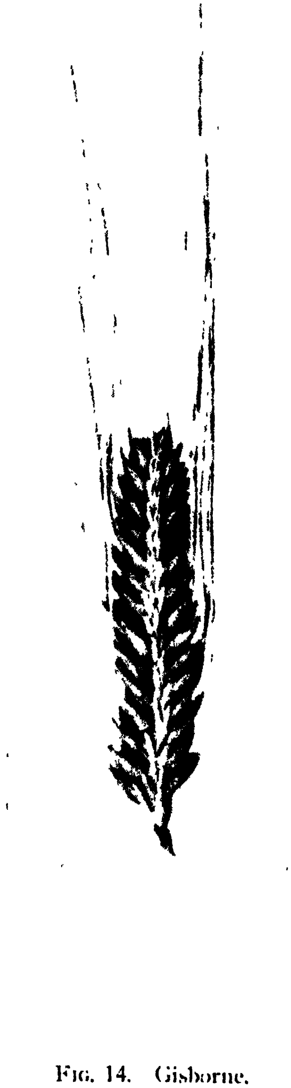

FIG. 14. Gisborne.

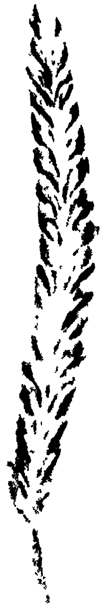

FIG. 15.—Goldthorpe Spratt.

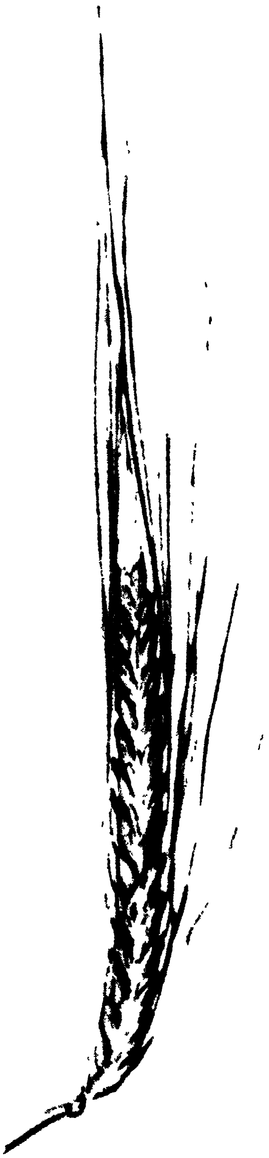

FIG. 16.—Kenia.

PLUMAGE ARCHER

The variety originated from a cross between Plumage and Archer made by Dr. Beaven in 1905. It was introduced to New Zealand in 1924 and became popular because of its strong straw and standing ability on heavy land where where it gives good yields of relatively good quality grain. Neck-break reduces yield considerably in some cases.

Description: Two-rowed spring barley; *H. zeocriton* L.; juvenile growth habit semi-erect; plant late, tall; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf medium long, medium wide, rolled, twisted; top of peduncle mostly unsheathed; spike dense, short, tapering, erect; awn long, barbed, pigmented; lemma with transverse crease at base, no teeth on lateral nerves; rachilla long haired; kernels white, long, uniform shape and size (Fig. 17).

PRYOR

Pryor is an early maturing Australian variety introduced into Nelson and Blenheim in 1937 and is now the predominant variety under the name of "Marlborough Chevallier". It is frequently confused with Kinver Chevallier, but is quite distinct in appearance and performance. Its early maturity suits the practice of late sowing, and also short, dry growing seasons. The straw is weak and the malting quality definitely poor.

Description: Two-rowed spring barley; *H. distichum* L.; juvenile growth habit erect; plant early, short; basal leaf sheaths non-hairy; base of culms pigmented, leaves medium long, medium wide; flagleaf medium long, narrow, not rolled, twisted; top of peduncle mostly unsheathed; spike lax, medium long, slight taper, nodding, awn long, barbed, not pigmented; lemma with depression at base, teeth on lateral nerves; rachilla short haired; kernels white, short, uniform shape and size (Fig. 18).

RESEARCH

Research was derived from a cross between Plumage Archer and Pryor made in Australia. It has rapidly become popular in the Ellesmere Springs district and the substantial areas grown since the 1945-46 season have provided good yields of less than average quality grain. It matures about 10 days earlier than Plumage Archer and stands well for direct heading. The straw is moderately strong.

Description: Two-rowed spring barley; *H. zeocriton* L.; juvenile growth habit semi-erect; plant early, midtall; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide, flagleaf medium long, narrow, not rolled, twisted; top of peduncle mostly unsheathed; spike dense, short, slight taper erect; awn long, barbed, not pigmented; lemma with transverse crease at base, no teeth on lateral nerves; rachilla long haired; kernels white, long, uniform shape and size (similar to Fig. 17).

SPRATT ARCHER

Spratt Archer originated from a cross between Irish Archer and Spratt made by Dr. Hunter in Ireland in 1908. It was introduced into New Zealand just before 1924, and is now one of the most important varieties in New Zealand. With short, strong straw, Spratt Archer produces good yields of excellent quality grain under most conditions.

Description: Two-rowed spring barley; *H. distichum* L.; juvenile growth habit semi-erect; plant late, short; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, narrow; flagleaf short, narrow, rolled, not twisted; top of peduncle sheathed; spike lax, long, paralled, nodding; awn long, barbed, pigmented; lemma with depression at base, no teeth on lateral nerves; rachilla long haired; kernels white, medium long, uniform shape and size (Fig. 19).

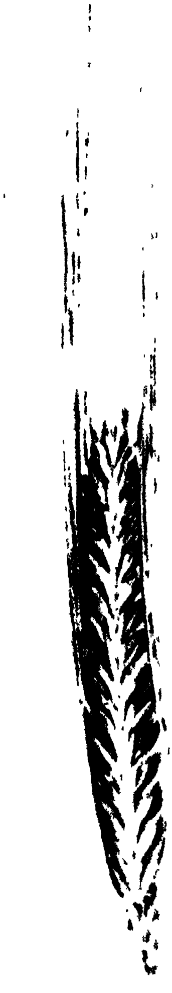

Fig. 17.—Plumage Archer.

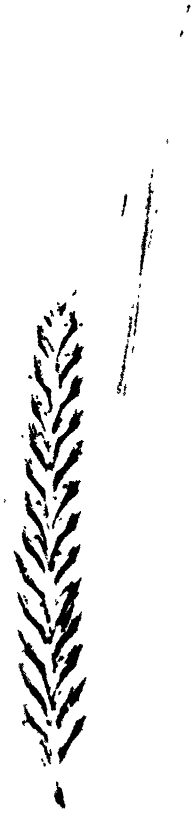

Fig. 18.—Pryor.

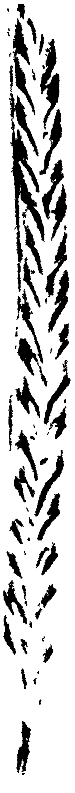

Fig. 19.—Spratt Archer

VICTORY

This variety was introduced from England in 1937, but differs from the European description in having a short-haired rachilla. Overseas reports also mention its good standing ability which is not evident in crops grown here. A few hundred acres have been grown in Canterbury.

Description: Two-rowed spring barley; *H. distichum* L.; juvenile growth habit semi-erect; plant midseason, midtall; basal leaf sheaths non-hairy; base of culms pigmented; leaves medium long, medium wide; flagleaf medium long, wide, not rolled, not twisted; top of peduncle mostly unsheathed; spike lax, long, parallel, nodding; awn long, barbed, pigmented; lemma with depression at base, no teeth on lateral nerves; rachilla short haired; kernels white, medium long, uniform shape and size (similar to Fig. 16).

SIX-ROW VARIETIES

CAPE

This is the most popular feed variety grown, occupying about 10 per cent of the total barley area. It has been grown in New Zealand for many years and originated in the Cape of Good Hope district in South Africa. The straw is weak and neck-break is common.

Description: 'Six-rowed spring barley; *H. vulgare* L.; juvenile growth habit semi-erect; plant early midtall; basal leaf sheaths with a few hairs; base of culms not pigmented; leaves medium long, wide, flagleaf medium long, wide, rolled, twisted; top of peduncle usually unsheathed; spike lax, short, parallel, erect; awn long, barbed, not pigmented; lemma with transverse crease at base, teeth on lateral nerves; rachilla short haired; kernel blue, long, varying shape and size (Fig. 20).

BLACK SKINLESS

Black Skinless is grown mainly to provide grazing in the autumn and winter. It has weak straw and is subject to neck-break. About 6 per cent of the barley area is stated to be in this variety. Its origin was probably in the northern region of India.

Description: Six-rowed spring barley; *H. vulgare* L.; juvenile growth habit erect; plant early, midtall; basal leaf sheaths non-hairy; base of culms not pigmented; leaves medium long, wide; flagleaf medium long, wide, rolled, not twisted; top of peduncle usually unsheathed; spike lax, short, tapering, nodding; awn long, barbed, pigmented; lemma with depression at base, teeth on lateral nerves; rachilla long haired; kernel purple-black, short, varying shape and size.

NEWAL

This variety was introduced into New Zealand in 1937 from the University of Alberta, Canada. A few crops have been grown in recent seasons in Canterbury. The straw is medium strong but severe shaking occurs even before the grain is ripe. Newal produces a very fast, bulky greenfeed crop which will not, however, stand up to repeated grazing.

Description: Six-rowed spring barley; *H. vulgare* L.; juvenile growth habit erect; plant early, midtall; basal leaf sheaths non-hairy; base of culms not pigmented; leaves medium, long, wide; flagleaf medium long, wide, rolled, not twisted; top of peduncle usually unsheathed; spike lax, medium long, parallel, nodding; awn long, smooth, not pigmented; lemma with transverse crease at base, no teeth on lateral nerves; rachilla long haired; kernel white, long, varying shape and size (Fig. 21).

WONG

Wong is a hybrid barley produced in China, and now grown as a winter barley in the north-eastern states of America. It shows promise of being useful for winter grazing. The straw is strong and erect.

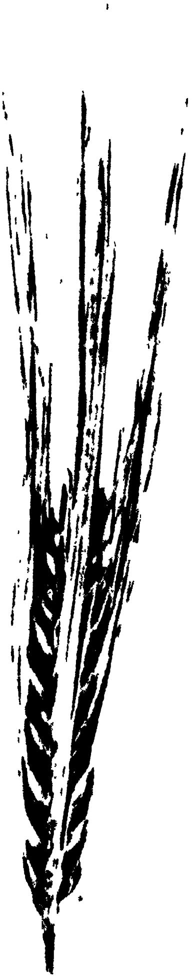

FIG. 20.—Cape.

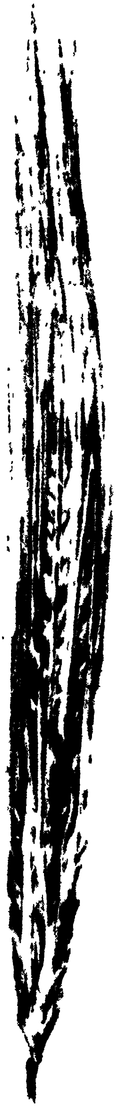

FIG. 21.—Newal

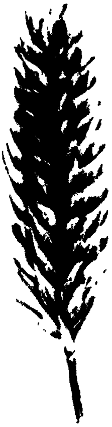

FIG. 22.—Wong.

Description: Six-rowed winter barley; *H. hexastichum* L.; juvenile growth habit prostrate; plant late, midtall; basal leaf sheaths hairy; base of culms not pigmented; leaves medium long, medium wide; flagleaf medium long, medium wide, rolled, not twisted; top of peduncle usually unsheathed; spike dense, short, parallel, erect; awn very short, barbed, not pigmented; lemma with depression at base; teeth on lateral nerves; rachilla long haired; kernels pale blue, medium long, varying shape and size (Fig. 22).

PREFECT

Prefect was obtained from the Plant Breeding Institute, Cambridge, England, in 1945. It is a recently selected hybrid barley from a cross between Praecox (a European six-row winter barley) and Spratt Archer. The straw is very long but is moderately strong and stands well. Prefect is a winter hardy grazing barley that produces a good plump feed grain.

Description: Six-rowed spring barley; *H. vulgare* L.; juvenile growth habit prostrate; plant late, tall; basal leaf sheaths non-hairy; base of culms not pigmented; leaves medium long, medium wide; flagleaf medium long, medium wide, rolled, twisted; top of peduncle usually unsheathed; spike lax, medium long, parallel, nodding; awn long, barbed, not pigmented; lemma with depression at base, teeth on lateral nerves; rachilla short haired; kernels white, long, varying shape and size.

IDENTIFICATION KEYS

JUVENILE STAGE

A. Erect habit.	
I Leaf sheath non-hairy	
(a) Very wide leaves	
(i) No pigment on culms	**Newal**
(b) Medium wide leaves	
(ii) Pigment on culms	**Pryor**
B. Semi-erect habit.	
I. Leaf sheath non-hairy.	
(a) Very wide leaves.	
(i) No pigment on culms	**Black Skinless**
(b) Medium wide leaves	
(ii) Pigment on culms	**Chevallier** **Culture 9** **Gisborne** **Golden Archer** **Goldthorpe Spratt** **Kenia** **Plumage Archer** **Research** **Victory**
(c) Narrow leaves.	
(ii) Pigment on culms.	**Spratt Archer**
II. Leaf sheath hairy.	
(a) Very wide leaves.	
(i) No pigment on culms.	**Cape**
C. Prostrate habit.	
I. Leaf sheath non-hairy.	
(b) Medium wide leaves.	
(i) No pigment on culms.	**Prefect**
(ii) Pigment on culms.	**Pioneer**
II. Leaf sheath hairy.	
(b) Medium wide leaves.	
(i) No pigment on culms.	**Wong**

POST EMERGENCE PERIOD

A. Six-rowed.	
I. No pigment on spike.	
(a) Long haired rachilla.	
(i) Dense eared	
Spike erect	**Wong**
(ii) Lax eared.	
Spike nodding.	**Newal**
(b) Short haired rachilla.	
(ii) Lax eared	
Spike erect	**Cape**
Spike nodding	**Prefect**
II Pigment on lemma	
(a) Long haired rachilla	
(ii) Lax eared.	
Spike nodding.	**Black Skinless**
B. Two-rowed	
I. No pigment on spike	
(a) Long haired rachilla	
(i) Dense eared	
Spike erect.	**Research**
(b) Short haired rachilla.	
(ii) Lax eared	
Spike nodding.	**Pryor**
II Pigment on sterile lateral spikelets and awn tips	
(a) Long haired rachilla	
(i) Dense eared	
Spike erect.	**Goldthorpe Spratt** **Plumage Archer**
(ii) Lax eared	
Spike nodding	
Peduncle invariably sheathed.	**Culture 9** **Spratt Archer**
Peduncle occasionally sheathed.	**Golden Archer**
Peduncle usually unsheathed.	**Kenia** **Pioneer**
(b) Short haired rachilla.	
(i) Dense eared	
Spike erect	**Gisborne**
(ii) Lax eared.	
Spike nodding	**Chevallier** **Victory**

GRAIN CHARACTERS

A. Covered grain.	
I. Short haired rachilla	
(a) Depression at grain base.	
(i) Even shape and size	
No teeth on lateral lemma nerves	**Chevallier** **Gisborne** **Victory**
Teeth on lateral lemma nerves.	**Pryor**
(ii) Uneven shape and size.	
Teeth on lateral lemma nerves.	**Prefect**
(b) Transverse crease at grain base.	
(ii) Uneven shape and size	
Teeth on lateral lemma nerves.	
Blue colour.	**Cape**
II. Long haired rachilla.	
(a) Depression at grain base	
(i) Even shape and size	
No teeth on lateral lemma nerves.	**Culture 9** **Golden Archer** **Pioneer** **Spratt Archer**
Teeth on lateral lemma nerves.	**Kenia**
(ii) Uneven shape and size.	

Teeth on lateral lemma nerves. Pale blue colour.	**Wong**
(b) Transverse crease at grain base.	
(i) Even shape and size. No teeth on lateral lemma nerves.	**Goldthorpe Spratt** **Plumage Archer** **Research**
(ii) Uneven shape and size. No teeth on lateral lemma nerves.	**Newal**
B. Skinless grain. Purple black colour.	**Black Skinless**

One realizes that in work of this kind it is difficult to avoid errors and any modification or correction would be welcomed by the writer.

ACKNOWLEDGMENTS

Acknowledgment is made to Mr R. C. Blackmore, Visual Aids Officer, Canterbury Agricultural College, for preparation of the photographs, and also to Miss M. E. Fairmaid, Agronomy Division, for the preparation of Figs. 3 and 5.

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A NOTE ON MORTALITY AND FERTILITY IN A NEW ZEALAND ROMNEY MARSH STUD-FLOCK IN 1938—THE SEASON OF THE FACIAL ECZEMA OUTBREAK

By H. GOOT, Massey Agricultural College, Palmerston North.

(Received for publication, 1st June, 1949)

Summary

The most severe outbreak of facial eczema in sheep in New Zealand occurred in the autumn of 1938 (Cooper and Walker 1940, Cunningham *et al.* 1942, and Levy 1942). As there is little, if any, factual information on losses incurred in facial eczema epidemics, a short table is presented pertaining to a stud flock which was affected by the 1938 outbreak. The stud concerned, called Stud B, has been described before (Goot 1946)

Table I shows the percentage: (i) of ewes which died or were missing *between mating and docking* (ewes which died at parturition without giving birth to a lamb are also included in this column); (ii) of dry and aborting ewes (some ewes whose lambs were stillborn or died soon after birth are probably included in this column); (iii) of multiple births; and (iv) of lambs docked.

Even allowing for a considerable margin of error in these data, there is little doubt that the stud flock was severely affected in the 1938 season The average mortality and percentage of dry ewes increased between three and four times. On the other hand, the incidence of multiple births and the percentage of lambs docked decreased by one-half. It is of interest to note that the relative decrease in percentage of lambs docked is remark-

TABLE I

Age-group	No of Ewes Put to Ram	Ewes Dead or Missing [1] Per cent	Ewes Dry or Aborting Per cent	Multiple Births. Per cent	Lambs Docked Per cent
		1938 Facial Eczema Year			
2-tooth	67	19.4	41.8	9.0	41.8
4-tooth	66	10.6	45.5	16.7	54.5
6-tooth	54	16.7	33.3	24.1	63.0
F.M. and over	105	24.8	31.4	27.6	62.9
Total	292	18.8	37.3	20.2	56.2
		1941-45 Seasons			
2-tooth	617	2.1	17.8	20.6	84.3
4-tooth	431	3.5	10.2	33.9	109.7
6-tooth	353	6.5	4.8	45.6	123.5
F.M. and over	492	7.9	6.7	50.0	119.9
Total	1,893[2]	4.8	10.4	35.9	106.7

[1] Between mating and docking.

[2] Total number of ewes in 5 year period.

All percentages based on number of ewes put to the ram ably even in all age-groups, although the factors responsible varied considerably in the different age-groups. In the 2-tooths, for example, the mortality was over nine times heavier than in seasons free of facial eczema, but figures for dry and aborting ewes were only about twice as high; almost the reverse situation obtains in 6-tooths.

It is emphasized that this note is based on one flock, and it may or may not be typical of the situation obtaining generally.

Thanks are due to Mr E. A. Clarke for reading critically the manuscript and for helpful suggestions.

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STUDIES ON SOME NEW ZEALAND ROMNEY MARSH STUD-FLOCKS:

PART III. TUPPING SEASON

By H. GOOT, Massey Agricultural College, University of New Zealand, Palmerston North, New Zealand.

(*Received for publication, 6 February 1949*)

Summary

(1) The following aspects of the tupping season have been examined. Distribution of the occurrence of first and last oestrus periods within the tupping season; duration of oestrus period and dioestrus cycle; repeatability of the occurrence of the first oestrus in successive tupping seasons; mating of rams by hand service.

(2) The investigation indicated that: (a) the first oestrus within the tupping season occurred from the second week in March to the second week in May; (b) the last oestrus in the tupping season depended mainly on the average number of tuppings per ewe; (c) the duration of oestrus ranged from one to four days; (d) the length of the dioestrus cycle ranged from 4 to 55 days with a mean of 17.2 days, and in 93 per cent. of cases the range was between 14 and 19 days; (e) no statistically significant differences in the length of the dioestrus cycles could be attributed to either age or season; (f) in the case of the rams during the tupping season, from a half to three-quarters of the days were spent either idly or with one, two, or three matings daily; (g) the average number of tuppings per ewe was 1.3.

INTRODUCTION

IN a previous paper the author (Goot, 1946b) dealt with the age composition of a stud flock prior to the tupping season. In this paper, certain data relating to the tupping season, which is herein defined as that part of the sexual season during which the rams are run with the ewes, are presented and analysed. The scope of the investigation was necessarily conditioned by the fact that most of the data on which the paper is based was recorded by a stud-master for his own use and not expressly for the purpose of making this study possible.

As breeders have found that stud ewes in the Manawatu district seldom come on heat during the first week of March (Buchanan 1945), the rams or the 'teasers' are not turned out until between the 6th and 10th of March. As none of the 1,418 ewes, in all the seven years under review, was recorded as having been tupped before 11 March, this date was chosen for all the years and age-groups as the first day of the tupping season.

Occurrence of the First Oestrus

The percentages of ewes exhibiting within a week, their first heat in the tupping season were tabulated separately for each age-group and year in Tables I and II. Data for the years 1943 and 1944 were not available because hand-service was not carried out in these seasons. The average proportion of ewes exhibiting the first oestrus in the tupping season was three per cent in the first week, rising rapidly to 16 per cent in the second, 30 per cent in the third and 31 per cent in the fourth; thereafter it fell abruptly to less than 10 per cent in each of the subsequent two weeks and less than two per cent in each of the last two to three weeks. The time interval elapsing between the first and last ewe coming on heat might appear to be rather great, but reference to work of similar nature, although based mostly on the sexual season, e.g., McKenzie and Terrill (1937), Schott *et al.* (1939), Walker (1943), and Hammond (1944) shows that it is not atypical. It should be stressed, however, that nearly 60 per cent of ewes, on the average, come on heat during the third and fourth weeks of the tupping season, i.e., the last week of March and the first week of April. In this respect the differences between the age-groups (Table I) ranged from 52 per cent for 2-tooth to 67 per cent for 6-tooth, and between the years (Table II) from 47 per cent in 1939 season to 82 per cent in 1942 season.

The percentage ot ewes which came on heat up to the end of the fifth week of the tupping season is also important, as it marks the approximate completion of the second 'colour' round of the sires.*

It will be seen that nearly 89 per cent of all ewes, on the average, came on heat within the first five weeks of the tupping season. The differences between age-groups ranged from 82 per cent for 2-tooth to 94 per cent for full-mouth and 5½-year-old ewes, and between the years, from 69 per cent in the 1939 season to 99 per cent in the 1942 season.

Although the differences between seasons were more pronouncd than between the age-groups, yet in the latter a distinct trend could be discerned. The 2-tooth and 6½-year-old and older ewes were slower in coming on the first heat during the tupping season than the remaining age-groups. As to seasonal differences it will be noted in Table II that the onset of the tupping season in 1937 and 1938 was retarded and the season started, rather atypically, at a very high level, in the second week. In all the other years, however, the first ewe was tupped between the 11th and 17th March, usually nearer the latter date. The effect of seasonal conditions, however, is most pronounced during the first five weeks of the tupping season, and especially during the peak period, viz., last week of March and first week of April. Season influenced but little the time interval between the first ewe and the last coming on heat for the first time in the tupping season, the difference being only one week. Thus it seems that whether a season is, what a breeder calls, a good or a bad one depends, apart from the fertility of rams, on the way the ewes come on heat during those five weeks and particularly on the peak period of the tupping season.

The distribution of the occurrence of the first oestrus within the tupping season can also be classified in 17-day intervals corresponding to the mean

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* The brisket of sires are raddled to mark the ewes which are tupped, the colour of the raddle being usually changed on every 17th or 18th day, and thus each 'colour' round approximates the average length of the oestrus cycle. (See also Goot 1946a.)

TABLE I. DISTRIBUTION OF THE OCCURRENCE OF THE FIRST OESTRUS WITHIN THE TUPPING SEASON, IN EWES OF DIFFERENT AGES

Percentages based on total tuppings in 7 seasons

No. of ewes Age.		392 2-tooth	307 4-tooth	258 6-tooth	283 Full-mouth and 5½ years.	178 6½ years and over.	Grand Total.	
Weeks.		%	%	%	%	%	No.	%
No.	Date.							
1.	11.3 - 17.3	3.6	2.3	1.6	4.2	2.8	42	3.0
2.	18.3 - 24.3	13.0	12.7	16.3	17.7	24.2	225	15.9
3.	25.3 - 31.3	30.6	30.3	25.2	32.9	28.7	422	29.8
4.	1.4 - 7.4	22.2	32.3	41.8	32.2	29.2	437	30.8
5.	8.4 - 14.4	13.0	6.8	8.9	7.4	6.7	128	9.0
6.	15.4 - 21.4	10.2	11.4	4.6	4.2	7.3	112	7.9
7.	22.4 - 28.4	2.8	2.6	1.6	- -	1.1	25	1.7
8.	29.4 - 5.5	4.3	1.6	- -	1.4	—	26	1.8
9.	6.5 - 12.5	0.3	- -	- -	—	—	1	0.1
	Total	100.0	100.0	100.0	100.0	100.0	1,418	100.0

TABLE II. DISTRIBUTION OF THE OCCURRENCE OF THE FIRST OESTRUS WITHIN THE TUPPING SEASON IN EWES IN DIFFERENT YEARS

No. of ewes Year.		155 1937	227 1938	231 1939	306 1940	207 1941	136 1942	156 1945	Grand Total.	
Weeks.		%	%	%	%	%	%	%	No	%
No.	Date									
1	11.3 - 17.3	-		1.3	3.6	6.8	1.5	7.7	42	3.0
2.	18.3 - 24.3	31.6	23.8	7.4	12.1	12.0	11.0	18.0	225	15.9
3	25.3 - 31.3	32.3	23.8	20.8	39.5	17.4	45.6	32.7	422	29.8
4.	1.4 - 7.4	25.2	28.6	26.0	23.9	47.3	36.8	33.3	437	30.8
5.	8.4 - 14.4	6.5	10.6	13.0	8.8	11.6	3.7	5.1	128	9.0
6.	15.4 - 21.4	2.6	7.9	19.0	11.1	3.9	0.7	1.9	112	7.9
7.	22.4 - 28.4	0.6	4.0	5.1	0.3	0.5	0.7	- -	25	1.7
8	29.4 - 5.5	0.6	1.3	7.4	0.7	0.5	-	1.3	26	1.8
9	6.5 - 12.5	0.6							1	0.1
	Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	1,418	100.0

length of the oestrus cycle. The following are the percentages thus computed: 11 to 27 March, 30.7; 2 March to 13 April, 56.3; 14 to 30 April, 11.7; and 1 to 17 May, 1.3.

OCCURRENCE OF THE LAST OESTRUS

Those ewes which do not conceive at the first heat period in the tupping season come on heat again until they are either in-lamb or the rams are withdrawn. Therefore, the shape of the curve of the last tupping depends on the fertility of both sexes and its variations throughout the season, on the duration of the tupping season and on the distribution of the time of occurrence of the first oestrus within the tupping season.

In Fig. 1 the distributions of the first and last oestrus periods in the tupping season are compared. These curves are based on the records of rams whose average number of tuppings per ewe varied from 1.1 to 1.8. Generally it may be said that the similarity of these two curves decreases with the increases in the average number of tuppings per ewe. However, it should be stressed that Fig. 1 must not be regarded as showing the only possible relationship between these curves, because the average number of tuppings per ewe does not give any direct indication of the variations in fertility throughout the tupping season.

The curve of last oestrus indicates approximately the distribution of lambing. This will be discussed in some detail in a later paper.

OESTRUS PERIOD

Oestrus was indicated by the ewe standing for the teaser, but its duration could be ascertained for the 1945 season only, as in this year every endeavour was made to allow the ewe to be served in the morning and evening of each day as long as she would accept the ram (Goot 1946a).

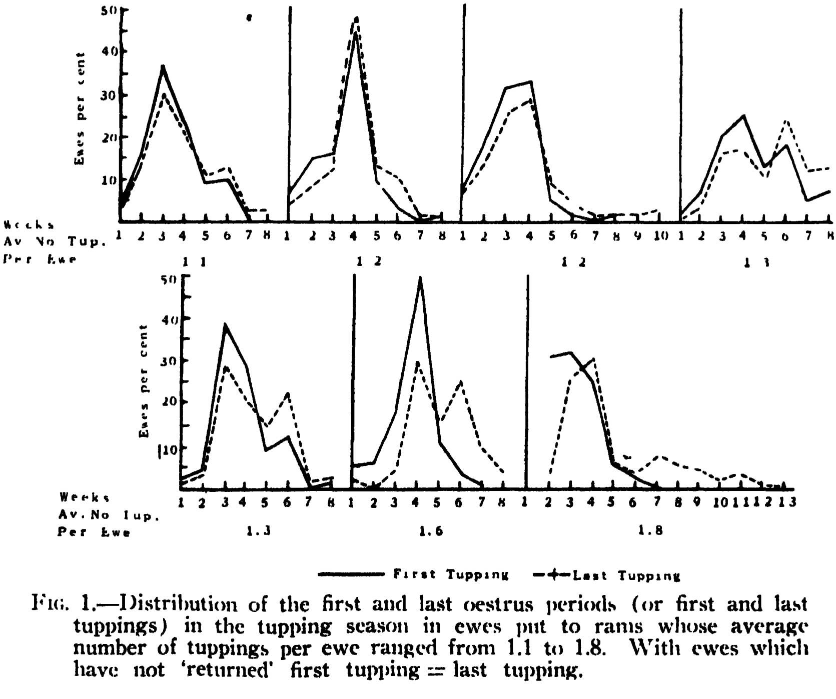


FIG. 1.—Distribution of the first and last oestrus periods (or first and last tuppings) in the tupping season in ewes put to rams whose average number of tuppings per ewe ranged from 1.1 to 1.8. With ewes which have not 'returned' first tupping = last tupping.

TABLE III LENGTH OF OESTRUS PERIOD IN EWES IN 1945 SEASON

Age-Group.	Length of Oestrus Period Less than 1 day.	1 day	2 days	3 days	4 days	Total
	%	%	%	%	%	No
2-tooth	1.1	69.0	24.1	4.6	1.1	87
4-tooth	6.5	77.4	12.9	3.2		31
6-tooth		57.7	38.4	3.8		26
Full-Mouth to 5½ years		51.4	45.7	2.9		35
6½ years and over		100.0				8
Total No	3	125	51	7	1	187
%	1.6	66.9	27.3	3.7	0.5	100.0

Table III gives the duration of 187 oestrus periods for 156 ewes of different ages. The individual periods were classified for length as follows: (1) less than one day ewes tupped by the teaser but refusing to stand for the ram when brought in; (2) one day ewes served on one day and refusing service on the next morning; (3) two days ewes served on two consecutive days and refusing service on the third morning, and so on. Obviously, measuring the length of oestrus in days cannot be very precise, and therefore a considerable margin of error must be allowed for (McKenzie and Terrill 1937, Kelley 1937, and others).

The duration of oestrus ranged from less than one day to four days. There were less than two per cent of periods shorter than one day, 67 and 27 per cent lasting respectively one and two days, and four per cent lasting more than two days.

The results obtained by other workers (Roux 1936, McKenzie and Terrill 1937, Kelley 1937, Kirillov 1938, and many others) show considerable variations in the length of oestrus period in different breeds, individuals, ages, seasons, and localities. The only previous observation on Romneys in this country indicated an oestrus period of 1.47 + 0.7 days (Walker 1943).

DIOESTRUS CYCLE

The periodicity of oestrus, or the length of dioestrus cycle was calculated from the date of the first hand service within an oestrus period, to the date of the first hand service within the next oestrus period. In this way 575 dioestrus cycles were ascertained and the relevant data are set out in Table IV. These figures are similar to those published by other workers (Milowanow 1932, Roux 1936, McKenzie and Terrill 1937, and many others), and for Romneys in this country by Gill (1933), Dry (1933), Webster (1937) and Walker (1943).

Differences in the length of dioestrus cycles due to age of ewes were found to be statistically not significant, a conclusion reached previously by McKenzie and Phillips (1931) and McKenzie and Terrill (1937). Similarly, the differences between the means of the years were found to

Table IV. Duration of Dioestrus Cycle in Ewes in Different Years

Year.	No. of cycles.	Mean ± S E	Restricted Mean *	Mode	Range	Per cent within 15-18 days.	Per cent. within 14-19 days.
		Days.	Days	Days	Days		
1937	148	17.86 ± 0.39	16.98	17	9 - 55	85.7	89.7
1938	188	16.88 ± 0.25	16.76	17	4 - 48	87.8	94.2
1939	52	16.81 ± 0.49	16.48	16	6 - 34	92.4	92.4
1940	53	17.42 ± 0.63	16.36	17	14 - 39	79.2	94.4
1941	71	16.63 ± 0.19	16.74	17	10 - 22	87.3	95.7
1942	36	16.58 ± 0.24	16.59	16	12 - 21	88.9	94.4
1945	27	17.70 ± 1.51	15.06	15	13 - 53	77.8	85.2
Total	575	17.17 ± 0.17	16.68	15 - 17	4 - 55	86.4	92.7

* Based on cycles within 14 to 19 days.

be statistically not significant, but further investigations of this aspect would seem desirable in view of the conflicting results obtained by other workers (Grant 1934, Elpatjevskii 1934, McKenzie and Terrill 1937, and Briggs *et al.* 1942).

As shown in Table IV, 92.7 per cent of dioestrus cycles fall within 14 to 19 days, but this varies in different seasons. Webster (1937) found amongst ewes from New Zealand Romney flocks that 95 per cent of cycles fell within this range. Assuming, after McKenzie and Terrill (1937), that this restricted range can be regarded as 'normal,' 7.3 per cent of cycles, being outside this range, may be classified as 'abnormal.' The following are the percentages of 'abnormal' cycles for each age-group: 2-tooth, 8.3; 4-tooth, 4.0; 6-tooth, 8.0; full mouth and 5½-year-old, 7.6; and 6½-year-old and over, 8.9. McKenzie and Terrill (1937) found the incidence of 'abnormal' cycles in various breeds to be 9.6 per cent (see also Kelley 1937).

McKenzie and Terrill (1937) believe that cycles shorter than normal may result from an early regression of the *corpus luteum*, and those recurring after an interval of 3 to 6 and 19 to 23 days might be due to the failure of ovulation and subsequent luteinization. Very long cycles

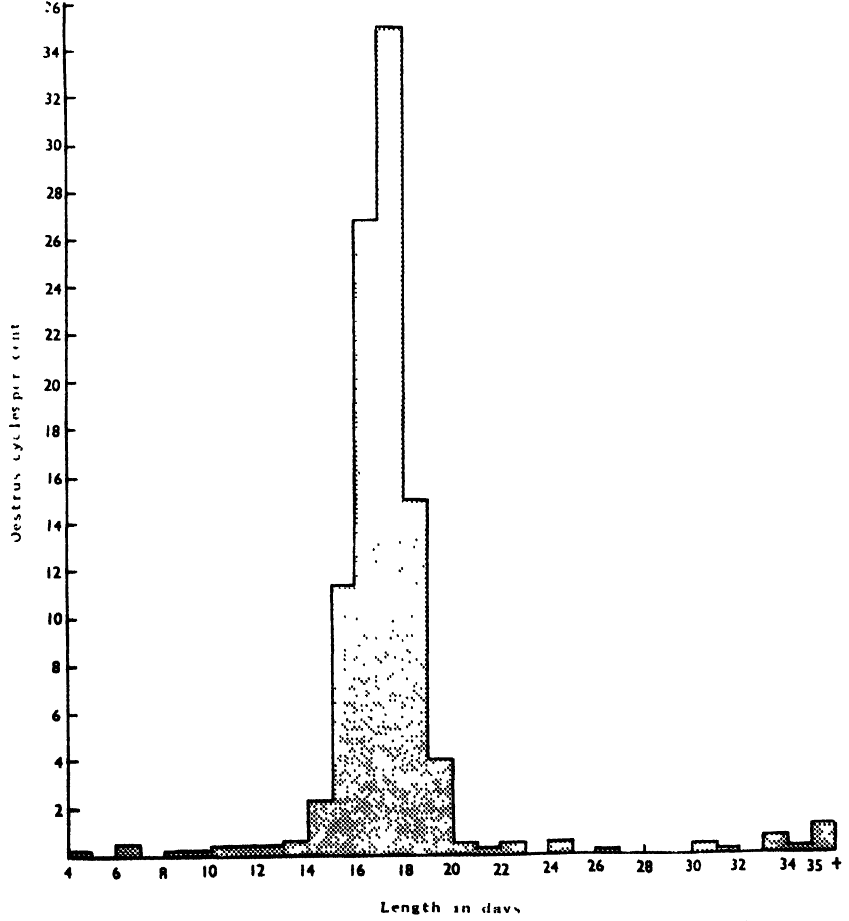


FIG. 2.—Frequency distribution per cent of the length of oestrus cycle.

may be caused by death of the embryo, undetected oestrus or ovulation without oestrus, the latter giving rise to silent heat or weak oestrus, and its length is the sum of two, three or more normal cycles. Silent heat is rather prevalent in Merino (Quinlan *et al.* 1941, and Kelley and Shaw 1943); in the present data, it does not appear to exceed two per cent.

REPEATABILITY OF OCCURRENCE OF THE FIRST OESTRUS

The repeatability of the first oestrus period within the tupping season was measured by correlation technique and the relevant results are given in Table V. Although there were some ewes coming on heat relatively early or late on two or more occasions, the ewes, except in the case of four-tooth versus six-tooth, have not shown any consistency which could be regarded as statistically significant. These results seem to indicate that, at least in the present material, factors other than the heredity make-up of the individual ewes exert the greater influence on the date of the first oestrus within each tupping season. The present findings, however, should be regarded as merely exploratory. The only conclusion that can be put forward with any confidence is that culling ewes for late lambing on the evidence of one or two seasons should be discouraged.

TABLE V. REPEATABILITY OF THE OCCURRENCE OF THE FIRST OESTRUS IN THE TUPPING SEASON IN EWES, AS MEASURED BY COEFFICIENTS OF CORRELATION

	D.F.	r
2-tooth vs 4-tooth	149	0.1023
4-tooth vs 6-tooth	104	0.2142*
6 tooth vs Full mouth	91	0.1931
2-tooth vs $\frac{\text{4-tooth} + \text{6-tooth}}{2}$	77	0.1501
4-tooth vs. $\frac{\text{2-tooth} + \text{6-tooth}}{2}$	77	0.1448

* significant at 5 per cent point

MATING OF RAMS BY HAND-SERVICE

Although hand-service is probably of considerable importance in breeding stud sheep, there has been little, if any, study of the utilization of rams mated by hand-service under ordinary stud-farm conditions. The material here presented touches some of these matters and is based on the records of five rams which were hand-served in Stud B.

Number of Ewes per Ram

As hand-service is mainly employed as a means for increasing the number of progeny by a given sire, it follows, all other things being equal, that the minimum number of ewes given to a ram for hand-service would be at least equal to the maximum number of ewes put to a ram in ordinary paddock mating. This, as shown in Part II (Goot 1946b), is about 75 ewes per ram. The greatest number of ewes hand-served by one ram was about 180 (Table VI), the average being 144. The above figures are probably indicatory only, for much depends on the season, fertility, vigour, and age of individual rams, as well as on the stud master's breeding policy and skill. It appears, therefore, that in comparison with the paddock mating, the hand-service method can at least double or treble the number of ewes put to a ram.

TABLE VI. UTILIZATION OF RAMS DURING HAND-SERVICE SEASON

Sire		1/38	2/39	3/43	6/32	7/35
Year		1940	1941	1945	1940	1937
No. of Ewes served		179	131	156	101	155
No. of tuppings*		207	158	200	127	279
Average number of tuppings per ewe**		1.1	1.2	1.2	1.3	1.8
Tupping season in days		51	51	55	52	68
Working days		40	42	46	39	57
Idle days	No.	11	9	9	13	11
	%	21.6	17.6	16.4	25.0	16.2
Average number of tuppings per day		4.1	3.1	3.6	2.4	4.1
Average number of tuppings per working day		5.2	3.8	4.3	3.3	4.9
Maximum number of tuppings per day		13	18	12	9	15

* The figures include also repeated services within the oestrus period, hence the slight discrepancy with the figures given in Table VIII.

** The averages were calculated on the basis of one service per oestrus period.

Utilization of Rams

Some further information on the day-to-day activities of hand-served rams is also given in Table VI. The tupping season for the five rams lasted from 51 to 68 days, with an average of 55.4 days. However, services were not performed on every day; the actual 'working' days ranged from 39 to 57, with an average of 44.8 'working' days per season. Similarly, days on which no service was performed, because there were no ewes on heat, ranged from 9 to 13, with an average of 10.6 days per season, or, in other words, the rest period ranged from 16 to 25 per cent, with an average of 19 per cent of idle days per season.

Number of Tuppings per day

An example of day-to-day reproductive activities of a ram is given in Fig. 3. Similar graphs were obtained for all the other rams tabulated in Table VI, and although they are not strictly comparable, owing to differences in the number of ewes served, number of tuppings per ewe, age, season, etc., their most common features are: (1) the irregular character of the curves, indicating that the more intensive periods of tupping were interspaced with less intensive and/or rest periods; (2) the peak periods during which the rams were obliged to work more steadily and perform a maximum of from 9 to 18 services a day.

On the whole, the intensive periods, except during the peak period, were of short duration and well spaced. The average number of tuppings per day for all days ranged from 2.4 to 4.1 for individual rams, and for 'working' days from 3.3 to 5.2 with the mean values of 3.5 and 4.3 services per day and per 'working' day respectively. In view of the

TABLE VII. FREQUENCY OF TUPPINGS PER DAY, I.E., THE NUMBER AND PERCENTAGE OF DAYS DURING WHICH THE RAMS PERFORMED VARYING NUMBER OF SERVICES FROM 0 TO 18

Sire.*	Days.	No of tuppings per day																			Total Days.	Days with 0-3 tuppings
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1/38	No.	11	4	4	6	6	4	5	1	3	4	1			2						51	25
	%	21.6	7.8	7.8	11.8	11.8	7.8	9.8	2.0	5.9	7.8	2.0			3.9						100.0	49.0
2/39	No	9	13	10	6	3	3	1	1			1		1	1	1				1	51	38
	%	17.6	25.5	19.6	11.8	5.9	5.9	2.0	2.0			2.0		2.0	2.0	2.0				2.0	100.3	74.5
3/43	No	9	13	4	6	4	3	6	3	1	2		1	3							55	32
	%	16.4	23.6	7.4	10.9	7.4	5.4	10.9	5.4	1.8	3.6		1.8	5.4							100.0	58.3
6/32	No	13	16	5	4	4	1	1	4	2	2										52	38
	%	25.0	30.8	9.6	7.7	7.7	1.9	1.9	7.7	3.8	3.8										99.9	73.1
7/35	No	11	9	15	5	5	2	5	1	2	2	5	3	1		1	1				68	40
	%	16.2	13.2	22.0	7.4	7.4	2.9	7.4	1.5	2.9	2.9	7.4	4.4	1.5		1.5	1.5				100.1	58.8

* Number and year of birth of the sire

TABLE VIII AVERAGE NUMBER OF TUPPINGS PER EWE

Age of Dams.	Tuppings by Sires 4 36* and 5 38* (1939)					Tuppings by Sire 1 38* (1940)					Tuppings by Sire 6 32* (1940)				
	I.	II	III	Total	Aver per ewe	I	II	III	Total	Aver per ewe	I	II	III	Total	Aver per ewe
2-tooth	81	19	1	101	1.3	20	4	1	25	1.3	25	10	1	36	1.4
4-tooth	38	8		46	1.2	60	5	2	67	1.1	31	4	1	36	1.2
6-tooth	36	11	1	48	1.3	15	3		18	1.2	29	5		34	1.2
Full Mouth and 5½ years	55	16	2	73	1.3	40	4		44	1.1	16	4		20	1.3
6½ years and over	20	3		23	1.2	44	5		49	1.1	-		-	-	-
Total	230	57	4	291	1.3	179	21	3	203	1.1	101	23	2	126	1.3

Age of Dams.	Tuppings by Sire 2 39* (1941)					Tuppings by Sire 1 38* (1941)					Tuppings by Sire 2 39* (1942).				
	I	II	III	Total	Aver per ewe	I	II	III	Total	Aver per ewe	I	II	III.	Total	Aver per ewe.
2-tooth	34	14	3	51	1.5	15	6		21	1.4	43	16	1	60	1.4
4-tooth	16	2		18	1.1	9	4		13	1.4	25	5		30	1.2
6-tooth	35	2		37	1.1	24	13	2	39	1.6	5	1		6	1.2
Full Mouth and 5½ years	22	2		24	1.1	16	12	2	30	1.9	23	3		26	1.1
6½ years and over	24	3		27	1.1	12	6		18	1.5				-	--
Total	131	23	3	157	1.2	76	41	4	121	1.6	96	25	1	122	1.3

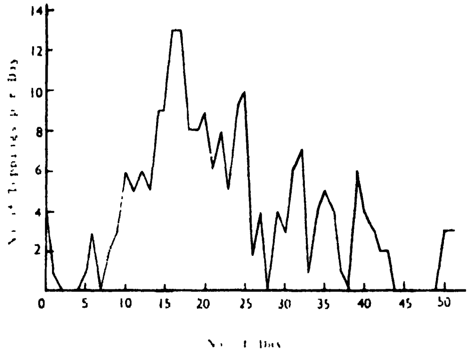
* Number and year of birth of the sire

TABLE VIII. (Continued)

Age of Dams.	Tuppings by Sire 8 35* (1942)					Tuppings by Sire 3 43* (1945).					Total Tuppings by all Sires.				
	I.	II.	III.	Total	Aver per ewe	I	II	III	Total	Aver per ewe.	I.	II	III.	Total.	Aver per ewe.
2-tooth	9	2	1	12	1.3	71	11	6	88	1.2	298	82	14	394	1.32
4-tooth	15	5	--	20	1.3	25	6	-	31	1.2	219	39	3	261	1.19
6-tooth	11	3		14	1.3	21	3	2	26	1.2	176	41	5	222	1.26
Full Mouth and 5½ years	5	3	--	8	1.6	31	4	-	35	1.1	208	48	4	260	1 25
6½ years and over	—	—	--		.	8	-		8	1.0	108	17	—	125	1.16
Total	40	13	1	54	1.4	156	24	8	188	1.2	1,009	227	26	1,262	1.25

* Number and year of birth of the sire

character of the curves the average is of little value. A truer perspective of the activities of a ram may be gained by recalculating the original figures on the basis of the number of days on which the rams performed services varying in number from 0 to 18. These data are summarized in Table VII and an example is given in Fig. 3b. It will be observed that a fair proportion of days is spent either idly or with one, two or three services per day. In fact, between half and three-quarters of all the days are spent in that way. On the other hand there were days during which a ram was used very intensively. As far as the writer is aware, there is little information available on the effect of the fertility of the ram of varying numbers of services daily. It would seem, therefore, highly desirable that further investigations be made in this direction.



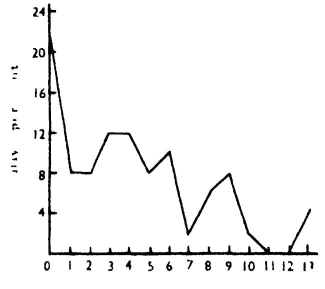


Fig. 3a.—Number of tuppings per day during the 1940 tupping season, ram 1/38 mated by hand-service to 178 ewes (See Table VI.)

Fig. 3b.—Frequency distribution per cent of tuppings for the same ram in the same season (See Table VII.)

Number of Tuppings per Ewe

Material available from Stud B on the average number of tuppings per ewe is summarized in Table VIII. Owing to the nature of the data the following reservations should be kept in mind:—

(a) One or more services per heat period were counted as one tupping; (b) it was not always possible to separate all the ewes allotted to each ram, a few 'returning' ewes being mated with other rams; (c) the word 'settle' does not necessarily mean that the ewe conceived. A ewe is considered 'settled' when she has not returned to the ram, or the ram has been withdrawn at the end of the tupping season.

The average number of tuppings per ewe, based on eight records of seven hand-served sires was 1.3.

The average number of tuppings per ewe calculated according to age of ewes ranged from 1.0 to 1.9. The differences between these age-group averages were statistically not significant.

Table VIII tabulates also the number of ewes served once, twice, and three times during the breeding season. Of the total of 1,009 ewes, 227 or 22.5 per cent were served twice and 26 or 2.6 per cent three times. Out of the 227 returning for the second time, 26, or 11.5 per cent, returned for the third time. In other words, 782 ewes, or 77.5 per cent, were settled after the first service and 201 or 88.5 per cent after the second.

Quinlan and Maré (1931) calculated the following percentages of South African Merinos fertilized in successive hand-services: first service, 50.2; second, 37.4; third, 48.9; fourth, 23.5; and fifth, 16.7. Roux and

Rensburg (1935) found abnormally large percentages of South African Merino ewes not 'settled' at the first service; viz., 32.1 per cent with two Border-Leicester rams; 37.9 per cent with two Ryeland rams; 70.6 per cent with two Southdown rams; and 12 per cent with one Merino ram. Kardymovich (1932) stated that 74 per cent of the Voloshian ewes were fertilized after the first mating.

The above figures (with the possible exception of the latter) refer to hand-service. Kelley (1937) found that 'paddock-mating' was significantly better than 'hand-service.'

No similar data, as far as the writer is aware, have been published in this country for Romney Marsh sheep, and therefore it is not possible to say how representative are the figures here presented. Further studies on the tupping season and factors affecting it, would seem desirable.

The lambing season will be discussed in a subsequent paper.

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